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(54) Title: METHOD FOR PREPARING OLIGONUCLEOTIDES

(57) Abstract: A method for preparing an oligonucleotide comprising the steps ofa) providing a 3 -protected compound having the formula: whereinB is a heterocyclic baseR2 is H, a protected 2 -hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4′- O2′methylen linkage R3 is OR′3, NHR″3, NR″3R‴3, a 3′-protected nucleotide or a 3′-protected oligonucleotide,R′3 is a hydroxyl protecting group,R″3, R‴3 are independently an amine protecting group, b) reacting said compound with a nucleotide derivative having a 5 -proctection group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bondc) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequencec1) capping preferably by reacting with a solid supported capping agentc2) oxidizing preferably by reacting the oligonucleotide with a solid supported oxidizing reagent d) removing the 5′ -protection group.

Method for preparing oligonucleotides

The present invention relates to a method for preparing oligonucleotides.

The synthesis of oligonucleotides has been the subject of investigations for a long period of time. Automated synthesis procedures have been developed and apparatus for the automated syntheses are commercially available. Most of these procedures have been developed for rather small quantities of oligonucleotides (in the range of mg). These amounts are sufficient for most investigational purposes.

Especially with the development of antisense therapeutics, large scale synthesis became a matter of considerable importance. Although relative large scale amounts of oligonucleotides have been obtained by scale-up of solid phase synthesis procedures, these technologies show major limitations especially high costs for reagents and materials, e.g. the solid phase bound starting oligonucleotide.

With scale-up, the reaction time of each step of the synthesis increases.

Furthermore oligonucleotides synthesis by standard solid phase synthesis results in a contamination of the desired full length compound by failure sequences arising from incomplete reaction during the synthesis cycle. At large scales the purification of the crude oligonucleotide involves complicated isolation and chromatographic purification of the final product.

In general, synthesis methods for oligonucleotides consist of a four-step procedure for the elongation of the oligonucleotide

1. Coupling
2. Capping
3. Oxidation
4. Deprotection of the protected hydroxyl group for the next reaction cycle.

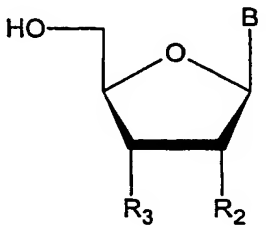
One object of the present invention is to provide a method for the preparation of oligonucleotides suitable for large scale (kilogram to tons) synthesis.

A further object of the present invention is to provide a method for the preparation of oligonucleotides avoiding complicated purification steps, especially chromatographic purifications, especially during synthesis cycles.

A further object of the invention is to provide a method for the preparation of oligonucleotides allowing an effective convergent synthesis.

In one embodiment this object is solved by a liquid phase synthesis method, comprising the steps of

a) providing a 3'-protected compound having the formula:



wherein

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4'-O2'methylen linkage

R_3 is OR'_3, NHR''_3, $NR''_3R'''_3$, a 3'-protected nucleotide or a 3'-protected oligonucleotide,

R'_3 is a hydroxyl protecting group,

R''_3, R'''_3 are independently an amine protecting group,

b) reacting said compound with a nucleotide derivative having a 5'-proctection group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bond

c) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequence

c1) capping, preferably by reacting with a solid supported capping agent

c2) oxidizing, preferably by reacting the oligonucleotide with a solid supported oxidizing reagent

d) removing the 5'-protection group.

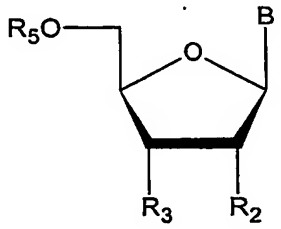
The method of the present invention is a solution phase synthesis wherein at least some of the reagents are solid supported. "Solid supported" covers covalently bound reagents and reagents bound to a solid support by ionic forces.

In a preferred embodiment, step d) is effected by treatment with a solid supported agent or removing the 5'-protection group with a removal agent followed

by addition of a solid supported scavenger or followed by extraction.

In most cases coupling occurs of a 5'-OH-synthon with a 3'-phosphorous-synthon. Alternatively coupling of a 5'-phosphorous-synthon with a 3'-OH-synthon is also possible. Therefore in a further embodiment the invention comprises a method comprising the steps of

a) providing a 5'-protected compound having the formula:



wherein

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4'-O2'methylen linkage

R_3 is OH, NH_2

R_5 is a hydroxyl protecting group, a 5'-protected nucleotide or a 5'-protected oligonucleotide

b) reacting said compound with a nucleotide derivative having a 3'-proctection group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bond

c) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequence

c1) capping, preferably by reacting with a solid supported capping agent

c2) oxidizing, preferably by reacting the oligonucleotide with a solid supported oxidizing reagent

d) removing the 3'-protection group.

In a preferred embodiment, step d) is effected by treatment with a solid supported agent or removing the 3'-protection group with a removal agent followed by addition of a solid supported scaenger or followed by extraction.

In further embodiments, it is possible to couple a 3′-phosphorous synthon with a 3′-OH synthon to form a non-natural 3′-3′-internucleosidic linkage. For the synthesis of non-natural 5′-5′-internucleosidic linkages it is possible to react a 5′-phosphorous synthon with a 5′-OH synthon. These non-natural internucleosidic linkages show increased nuclease resistance.

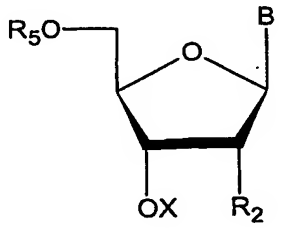
Step a)

B, the heterocyclic base can be a natural nucleobase or a modified one including a non-base residue. The natural nucleobasis are adenine, guanine, thymine, cytosine and uracil. In general these bases need protection groups during the synthesis. Suitable protected nucleobases are known to persons skilled in the art for example N-4-benzoylcytosine, N-6-benzoyl adenine, N-2-isobutiryl guanine, N-4-acetyl or isobutyril cytosine, N-6-phenoxyacetyl adenine, N-2-tert-butyl phenoxyacetyl guanine. Suitable non-base residues include for example Hydrogen, H leading to the 1′,2′-dideoxyribose (dSpacer from Glen Research) which can be used as linker or to mimic abasic sites in an oligonucleotide (Takeshita et al., J. Biol. Chem., 1987, 262, 10171).

Furthermore, it is also possible to use isomers of nucleosides such as L, D, α, ß and the like.

A suitable protection for the 2'-hydroxyl-group include but are not limited to tert-butyl dimethylsilyl (TBDMS), triisopropylsilyloxymethyl (TOM), fluorophenylmetoxypiperidinyl (FPMP).

Suitable protecting groups for the 3'-hydroxyl-group include but are not limited to tert-butyl dimethylsilyl (TBDMS), levulinyl, benzoyle. This compound is then reacted with a nucleotide derivative with a 3'-phosphorous-synthon. The nucleotide derivative preferably has the following formula



(Formula III)

wherein

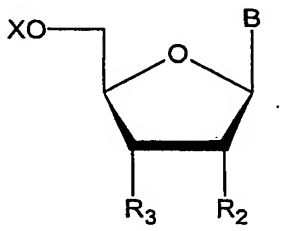
X is a P(III)-function

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4'- O2'methylen linkage

R_5 is a hydroxyl protecting group, a 5'-protected nucleotide or a 5'-protected oligonucleotide.

In the second embodiment, the nucleotide derivative preferably has the following formula



(Formula IV)

wherein

X is a P(III)-function

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4'- O2'methylen linkage

R_3 = OR'$_3$, NHR"$_3$, NR"$_3$R'"$_3$, a 3'-protected nucleotide or a 3'-protected oligonucleotide,

R'$_3$ is a hydroxyl protecting group,

R"$_3$, R'"$_3$ are independently an amine protecting group,

R'$_3$ is a hydroxyl protecting group, a 3'-protected nucleotide or a 3'-protected oligonucleotide

Step b): The coupling step

In step b) the coupling of the nucleotide or oligonucleotides occurs. The chemistry of the reaction depends on the type of activated phosphorous compound.

Several methods for coupling nucleotides are known. The most common methods are via phosphoramidite and via H-phosphonate. In each of these cases the phosphor is in an activated state which allows coupling with the free hydroxyl group of the other part.

In phosphoramidite chemistry (Beaucage et al., Tetrahedron, 1992, 48, 2223-2311: Beaucage and Caruthers in unit 3.3 of Current Protocols in Nucleic Acid Chemistry, Wiley) a nucleoside or oligonucleotide-3′-O-phosphoramidite where the P(III) phosphorus is substituted with a dialkylamine (phosphite activating group) and a phosphorus protecting group (including but not limited to 2-cyanoethyl, methyl) is reacted with 5′-hydroxyl nucleoside or oligonucleotide in presence of an activator to create a phosphite triester internucleosidic linkage.

In H-phosphonate chemistry (Froehler, Methods in Molecular Biology. Protocols for oligonucleotides and analogs, Humana Press, 1993, 63-80; Strömberg and Stawinski, in unit 3.4 of Current Protocols in Nucleic Acid Chemistry, Wiley) a nucleoside or oligonucleotide-3′-O-H-phosphonate is reacted with a 5′-hydroxyl nucleoside or oligonucleotide in presence of an activator to create a H-phosphonate diester internucleosidic linkage.

Suitable activators for the coupling step in phosphoramidite chemistry include, but are not limited to a solid support bearing a pyridinium salt, for example a solid support covalently linked to pyridine e.g. poly(vinyl)-pyridinium or the pyridinium is a counter ion of a cation exchange solid support. The cation exchange support can be a strong or a weak exchanger, for example a sulfonic acid or a carboxylic acid. The pyridinium salt can also be a substituted pyridinium salt, for example dichloropyridinium. It can further be a solid support bearing an optionally substituted azole (imidazole, triazole, tetrazole), or is the salt of a weak base anion exchange resin with a strong acid, or a weak cation exchange resin (carboxylic) in its protonated form (see US patent 5,574,146), or a solid support bearing an optionally substituted phenol (see W. Dabkowski and al., Tet Lett, 2000, 41, 7535-7539).

The use of imidazole is less preferred.

For the H-phosphonate method the suitable activators include, but are not limited to solid supports bearing a carboxylic acid chloride, sulfonic acid chloride, a chloroformate, a chlorosulfite or a phosphorochloridate or the respective Br-compounds. Further compounds are disclosed in WO 01/64702 A1, page 6, line 36 to page 8, line 5, incorporated by reference and CB Reese and Q Song, Nu-

cleic Acid Res., 1999, 27, 963-971.

Step c) Capping and Oxidizing

Capping is understood as a reaction wherein a reagent reacts with remaining protected compounds of step a). As the capping agent is preferably solid supported, the 3'-protected compound can be removed together with the solid supported capping agent.

For the capping step suitable reagents suitable capping agents include, but are not limited to activated acids for example carboxylic acid, chloride or sulfonic acid chloride, carboxylic acid bromide, azolide, substituted azolide, anhydride or chloroformate or phosphorochloridate, or a solid supported phosphoramidate, or a solid supported H-phosphonate monoester. The acid group is preferably an acid group covalently bound to a solid support. Commercially available cationic exchanger resins can be used as a starting material for synthesizing the solid supported carboxylic acids or sulfonic acids.

The oxidizing reaction is used to oxidize the P(III)-internucleotide bond to a P(V)-internucleotide bond. Capping can be performed before oxidizing and vice versa. Depending on the reagents capping and oxidizing may also be combined in one step.

In case of H-phosphonate chemistry, the oxidizing step is preferably done in every second or third cycle or at the end of synthesis only. In phosphoamidite chemistry removal of the excess of 5´ nucleoside oligonucleotide can be facsilated by a hydrolysis step, for example with water.

For the oxidizing step the oxidizing reagent can be any oxidizing reagent used for prior art solid phases, preferably in the form of solid supported agent, either covalently bound or bound by ionic forces. Suitable reagents are solid supported periodates, permanganates, osmium tetroxides, dichromates, hydroperoxides, substituted alkylamine oxides, percarboxylic acid and persulfonic acid.

These compounds are negatively charged, therefore they can be solid supported by a suitable ion exchanger for example an ion exchanger bearing ammonium groups. These substances could be bound to solid support consisting for example of an amino, alkyl amino, dialkyl amino or trialkyl amino anion exchanger.

In oligonucleotides synthesis for investigational purposes and especially for antisense therapeutics phosphorthioate analogs are used. In this case the oxidizing is a sulfurization. As a solid supported oxidizing reagent a solid supported sulfu-

rization reagent is used, for example a solid supported tetrathionate, a solid supported alkyl or aryl sulfonyl disulfide, a solid supported optionally substituted dibenzoyl tetrasulfide, a solid supported bis(akyloxythiocarbonyl)tetrasulfide, a solid supported optionally substituted phenylacetyl disulfide, a solid supported N-[(alkyl or aryl)sulfanyl] alkyl or aryl substituted succinimide and a solid supported (2-pyridinyldithio) alkyl or aryl.

Very preferred is a solid supported cyanoethylthiosulfate ($NC\text{-}CH_2\text{-}CH_2S\text{-}SO_3^-$), available according to the procedure in US 3,506,676 or a solid supported tetrathionate.

Step d) Deprotection

Suitable 5'-protection group include, but are not limited to trityl groups, preferably a dimethoxytrityl group (DMTr) or a monomethoxytrityl group (MMTr). These protection groups are used in conventional prior art solid phase oligonucleotides synthesis. Other suitable 5'-protection groups include, but are not limited to tert-butyl dimethylsilyl (TBDMS), levulinyl, benzoyle, fluorenemethoxycarbonyl (FMOC), the 9-phenylthioxanthen-9-yl (S-pixyl).

In the second embodiment, in step d) the 3'-protection group is removed. Suitable 3'-protection groups include, but are not limited to 3'-*O*-*tert* butyl dimethyl silyl (TBDMS), 3'-*O*-acetate, 3'-*O*-levulinyl groups. They can be removed by a solid-supported ammonium fluoride, solid-supported ammonium hydroxide or solid-supported hydrazine.

In step d) of the first embodiment, the 5'-protection group is removed. Thereafter the oligonucleotides can either be used or the oligonucleotide corresponds to the 3'-protected compound of step a) to repeat the cycle.

The use of solid supported reagents for the removal of the DMTr-protection group for a completely synthesized oligonucleotide has already been reported in US 5,808,042. The content of this document is incorporated by reference. Surprisingly the methods disclosed in US 5,808,042 can also be applied in a solution phase synthesis as described in the present application.

Suitable reagents are also disclosed in synthetic communications 24 (17) 1994, 2323-2428.

In step d) of the second embodiment, the 3'-protection group is removed. Thereafter the oligonucleotides can either be used or the oligonucleotide corresponds to the 5'-protected compound of step a) to repeat the cycle.

Step e): Repetition

In most cases the methods will be repeated at least once. When starting from monomeric oligonucleotides the method of the present invention will result in a dimer. Repeating the method of the present invention will elongate the dimer to a trimer. By repeating the method of the invention several times n-mers can be synthesized.

As the yield of a synthesis is not 100%, the overall yield of correct oligonucleotides decreases with the number of cycles. Depending on the yield of a single cycle, oligonucleotides can be synthesized of at least up to 100 nucleotides in sufficient yield, but longer oligonucleotides are also possible.

For most cases oligonucleotides having that size will not be needed. An antisense therapy oligonucleotides are normally in the range of 8-36 nucleotides, more preferably 12-30, most commonly in the range of the 16-26 nucleotides.

In contrast to prior art, convergent synthesis strategies are fully compatible with the synthesis method of the present invention. Convergent synthesis methods are methods wherein small oligonucleotides are synthesized first and the small oligonucleotides are then combined for synthesizing larger blocks. By this method the number of coupling reactions can be significantly reduced. Thereby the overall yield of the oligonucleotide is increased.

In prior art, each synthesis of a small oligonucleotide had to start from the solid support bound nucleic acid which was rather expensive. Therefore convergent synthesis strategies have not found much application in oligonucleotide synthesis.

Convergent synthesis has the further advantage, that the reaction product is essentially free of (n-1)mers. In prior art synthesis, the purification of oligonucleotides with a length of n from oligonucleotides with a length of n-1 is the most difficult in purification of the oligonucleotide. By convergent synthesis, these (n-1)mers are nearly avoided, because larger fragments are combined.

In a preferred embodiment, the method of the present invention uses dimers or trimers as the compounds in step a) and/or b).

During the synthesis cycles, reagents are mostly added in a solid supported form. These solid supported reagents are preferably removed after reaction or after each reaction step. Depending on the type of reagent it is in some cases possible to remove two or more of the solid supported reagents together.

In a preferred embodiment, coupling and at least final oxidizing steps are done by solid supported reagents.

As the synthesis is intended for the production of large amounts of oligonucleotides it is preferred that the solid supported reagent is recycled. This recycling is obviously easier if the solid supported reagents are removed separately after each reaction.

The solid supported reagents can be removed by methods like filtration or centrifugation. Because of the ease of handling, filtration is the preferred way of removing the solid supported reagents.

A very preferred reagent for the sulfurization is a solid supported anion exchange resin in complex with a tetrathionate having the formula S_4O_6, preferably a quaternary ammonium resin bearing tetrathionate as counter ion.

Purification

After the final synthesis cycle, it will in most cases be necessary to make one or more additional purification steps. Such purification steps for crude oligonucleotide synthesis products are known in prior art solid phase synthesis.

It is necessary to remove the remaining protection groups especially from the heterocyclic bases and optionally remaining protecting groups on the sugar or phosphorous backbone.

In a preferred embodiment, in case of the use of MMT or DMT as a protection group, the oligonucleotide is purified by binding to an ion exchanger and the protection group is removed while the oligonucleotide is bound to the exchanger. After removal of the protection groups, the oligonucleotide is released from the ion exchanger.

The invention will be further exemplified with the following examples.

Example 1

Synthesis of the dimer 5'-O-DMTr-T-T-3'-O-TBDMS cyanoethyl phosphite triester.

Coupling procedure of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite with 5'-OH-T-3'-*O*-TBDMS using the DOWEX 50W X8 pyridinium form.

Analytical scale.

5'-OH-T-3'-*O*-TBDMS (11 mg, 32.5 mmol) and 5'-*O*-DMTr-T-3'-cyanoethyl

phosphoramidite (41 mg, 55.25 mmol, 1.7 eq) are dissolved in anhydrous acetonitrile (550 ml). The solution is transferred under argon in a NMR tube containing the DOWEX 50W X8 pyridinium form (100 mg, 0.30 mmol $pyrH^+$, 9.2 eq). The reaction is followed by ^{31}P NMR. Before the NMR experiment deuterated acetonitrile (50 ml) is added. The yield is determined by ^{31}P NMR. After 3 h the desired dimer T-T phosphite triester is obtained with 100% of yield compared to 5'-OH-T-3'-*O*-TBDMS. The crude is a mixture of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite (^{31}P NMR (CD_3CN) δ 149.14,149.07, 14.7%), 5'-*O*-DMTr-T-T-3'-*O*-TBDMS cyanoethyl phosphite triester (δ 140.53, 70.2%), 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (δ 8.74, 15.1%).

Example 2

Synthesis of the dimer 5'-O-DMTr-T-T-3'-O-TBDMS cyanoethyl phosphite triester.

Coupling procedure of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite with 5'-OH-T-3'-*O*-TBDMS using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich).

Analytical scale.

5'-OH-T-3'-*O*-TBDMS (11 mg, 32.5 mmol) and 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite (41 mg, 55.25 mmol, 1.7 eq) are dissolved in anhydrous acetonitrile (550 ml). The solution is transferred under argon in a NMR tube containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (100 mg, 0.33 mmol tos^-, 10.3 eq). The reaction is followed by ^{31}P NMR. Before the NMR experiment deuterated acetonitrile (50 ml) is added. The yield is determined by ^{31}P NMR. After 1 h 45 the desired dimer T-T phosphite triester is obtained with 82% of yield compared to 5'-OH-T-3'-*O*-TBDMS. The crude is a mixture of 5'-*O*-DMTr-T-T-3'-*O*-TBDMS cyanoethyl phosphite triester (^{31}P NMR (CD_3CN) δ 140.54, 48.2%), 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (δ 8.77, 51.8%).

Example 3

Synthesis of the dimer 5'-OH-T-T-3'-O-TBDMS cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite with 5'-OH-T-3'-*O*-TBDMS using the DOWEX 50W X8 pyridinium form.

A solution of 5'-OH-T-3'-*O*-TBDMS (124 mg, 0.35 mmol) and 5'-*O*-DMTr-T-3'-

cyanoethyl phosphoramidite (441 mg, 0.59 mmol, 1.7 eq) in anhydrous acetonitrile (6 ml) is added to DOWEX 50W X8 pyridinium form (1 g, 3 mmol $pyrH^+$, 9.5 eq). The resulting mixture is shaken for 4 h 45. The reaction is followed by ^{31}P NMR and the yield is also determined by ^{31}P NMR. The desired dimer 5'-*O*-DMTr-T-T-3'-*O*-TBDMS cyanoethyl phosphite triester is obtained with 100% of yield compared to 5'-OH-T-3'-*O*-TBDMS. The crude is a mixture of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite (^{31}P NMR (CD_3CN) δ 149.17,149.10, 5.4%), 5'-*O*-DMTr-T-T-3'-*O*-TBDMS cyanoethyl phosphite triester (δ 140.57, 140.54, 68.3%), 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (δ 8.75, 8.71, 26.3%).

Sulfurization: The DOWEX 50W X8 resin is filtered off and the resulting solution is added to AMBERLYST A26 tetrathionate form (1.44 g, 2.44 mmol $S_4O_6^{2-}$, 7 eq.). The reaction is followed by ^{31}P NMR and the yield is also determined by ^{31}P NMR. After 20 h the desired dimer 5'-*O*-DMTr-T-T-3'-*O*-TBDMS cyanoethyl phosphorothioate triester is obtained with 97% of yield. The crude is a mixture of 5'-*O*-DMTr-T-3'- cyanoethyl thiophosphoramidate (^{31}P NMR (CD_3CN) δ 71.16, 4.0%), 5'-*O*-DMTr-T-T-3'-*O*-TBDMS cyanoethyl phosphorothioate triester (d 68.28, 68.23, 69.5%), 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (d 8.75, 8.71, 26.5%). MALDI-TOF MS (negative mode, trihydroxyacetophenone as matrix) ammonia treatment of an aliquot gives 5'-OH-T-T- 3'-*O*-TBDMS phosphorothioate diester: $[M-H]^-$ m/z_{exp} = 978.12, m/z_{calc} = 977.13.

Detritylation: The AMBERLYST A26 is filtered off and the solvent are evaporated. The crude is dissolved in 4ml of CH_2Cl_2/CH_3OH (7/3) and cooled in an ice bath. To this solution is added 1 ml of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 15 min at 0°C. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried (Na_2SO_4), evaporated, and purified on a silica gel column. The desired dimer T-T is eluted with CH_2Cl_2/CH_3OH (95/5). The appropriates fractions are collected and evaporated to give 230 mg of a white foam in a yield of 83% compared to 5'-OH-T-3'-*O*-TBDMS. ^{31}P NMR (CD_3CN) δ 68.29, 68.19. MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 730.46, m/z_{calc} = 730.82. The spectrophotometric purity (97%) is determined by HPLC at 260 nm.

Example 4

Synthesis of the trimer 5'-OH-T-T-T-3'-O-TBDMS cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite with the dimer 5'-OH-T-T-3'-*O*-TBDMS cyanoethyl phosphorothioate triester using the DOWEX 50W X8 pyridinium form.

A solution of 5'-OH-T-T-3'-*O*-TBDMS cyanoethyl phosphorothioate triester (230 mg, 0.31 mmol) and 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite (399 mg, 0.54 mmol, 1.7 eq) in anhydrous acetonitrile (8 ml) is added to DOWEX 50W X8 pyridinium form (1 g, 3 mmol $pyrH^+$, 9.5 eq). The resulting mixture is shaken for 5 h. The reaction is followed by ^{31}P NMR and the yield is also determined by ^{31}P NMR. The desired trimer 5'-*O*-DMTr-T-T-T-3'-*O*-TBDMS cyanoethyl phosphite triester is obtained with 100% of yield compared to the dimer 5'-OH-T-T-3'-*O*-TBDMS cyanoethyl phosphorothioate triester. The crude is a mixture of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite (^{31}P NMR (CD_3CN) δ 149.16,149.10, 17.7%), 5'-*O*-DMTr-T-T-T-3'-*O*-TBDMS cyanoethyl phosphite triester (δ 140.85, 140.68, 140.37, 140.30, d 68.07, 68.02, 68.3%), 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (δ8.7, 8.68, 14%).

Sulfurization: The DOWEX 50W X8 pyridinium form is filtered off and the resulting solution is added to AMBERLYST A26 tetrathionate form (1.3 g, 2.44 mmol $S_4O_6^{2-}$, 7 eq.). The reaction is followed by ^{31}P NMR and the yield is also determined by ^{31}P NMR. After 45 h the desired trimer 5'-*O*-DMTr-T-T-T-3'-*O*-TBDMS cyanoethyl phosphorothioate triester is obtained with 100% of yield. MALDI-TOF MS (negative mode, trihydroxyacetophenone as matrix) $[M-H]^-$ m/z_{exp} = 1297.89, m/z_{calc} = 1296.38 after 30 min of ammonia treatment to remove the cyanoethyl protecting group. The crude is a mixture of 5'-*O*-DMTr-T-3'- cyanoethyl thiophosphoramidate (^{31}P NMR (CD_3CN) d 72.04, 71.17, 14.0%), 5'-*O*-DMTr-T-T-T-3'-*O*-TBDMS cyanoethyl phosphorothioate triester (d 68.17, 68.12, 68.07, 67.96, 67.80, 67.58, 73.8%), 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (d 8.76, 8.71, 12.2%).

Detritylation: The AMBERLYST A26 is filtered off and the solvent are evaporated. The crude is dissolved in 4ml of CH_2Cl_2/CH_3OH (7/3) and cooled in an ice bath. To this solution is added 1 ml of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 45 min at 0°C. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried (Na_2SO_4), evaporated, and purified on a silica gel column. The desired trimer T-T-T is eluted with CH_2Cl_2/CH_3OH (95/5). The appropriates fractions are collected and evaporated to give 221 mg of a white foam in a yield of 63% compared to the dimer 5'-OH-T-T-3'-*O*-TBDMS cyanoethyl phos-

phorothioate triester. ^{31}P NMR (CD_3CN) δ 68.53, 68.38, 68.34, 67.74, 67.54. MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1103.91, m/z_{calc} = 1104.15. The spectrophotometric purity (93%) is determined by HPLC at 260 nm.

Example 5

Synthesis of the dimer 5'-OH-T-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-T-3'-phosphoramidite with 5'-OH-dA^{Bz}-3'-*O*-TBDMS using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich).

A solution of 5'-OH-dA^{Bz}-3'-*O*-TBDMS (176 mg, 0.38 mmol) and 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite (560 mg, 0.75 mmol, 2 eq) in anhydrous acetonitrile (6 ml) is added to poly(4-vinylpyridinum *p*-toluenesulfonate) (1.15 g, 3.84 mmol tos^-, 10.2 eq). The resulting mixture is shaken for 4 h 30 min. The reaction is followed by ^{31}P NMR and the yield is also determined by ^{31}P NMR. The desired dimer 5'-*O*-DMTr-T-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphite triester is obtained with 100 % of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-TBDMS. The crude is a mixture of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite (^{31}P NMR (CD_3CN) δ 149.10,149.05, 12.3%), 5'-*O*-DMTr-T-A^{Bz}-3'-*O*-TBDMS cyanoethyl phosphite triester (δ 140.52, 140.37, 50%), 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (δ 8.72, 8.69, 37.7%).

Sulfurization: The poly(4-vinylpyridinum *p*-toluenesulfonate) is filtered off and the resulting solution is added to AMBERLYST A26 tetrathionate form (1.55 g, 2.63 mmol $S_4O_6^{2-}$, 7 eq.). The reaction is followed by ^{31}P NMR. The reaction mixture is shaken for 24 h 30. The desired dimer 5'-*O*-DMTr-T-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphorothioate triester is isolated after filtration of the resin, evaporation of the solvent, and column chromatography (silica gel; CH_2Cl_2 / MeOH (50/1)). Yield: 325 mg, 0.28 mmol, 76%. ^{31}P NMR (CD_3CN) δ 68.34, 68.15. MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1144.22, m/z_{calc} = 1146.32.

Detritylation: The 5'-*O*-DMTr-T-dA^{Bz}- 3'-*O*-TBDMS cyanoethyl phosphorothioate triester is dissolved in 10 ml of CH_2Cl_2/CH_3OH (7/3) and cooled in an ice bath. To this solution is added 1 ml of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 35 min at 0°C. The reaction is washed with 20 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried (Na_2SO_4), evaporated, and purified on a silica gel

column. The desired dimer T-dA^{Bz} is eluted with CH_2Cl_2/CH_3OH (95/5). The appropriates fractions are collected and evaporated to give a white foam. Yield: 223 mg, 0.26 mmol, 71%. ^{31}P NMR (CD_3CN) δ 68.06, 67.89. MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 842.18, m/z_{calc} = 843.95; (negative mode, trihydroxyacetophenone as matrix) ammonia treatment of an aliquot gives 5'-OH-T-dA- 3'-*O*-TBDMS phosphorothioate diester: $[M-H]^-$ m/z_{exp} = 685.38, m/z_{calc} = 684.77.

Example 6

Synthesis of the trimer 5'-O-DMTr-dA^{Bz}-T-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl phosphoramidite with the dimer 5'-OH-T-A^{Bz}-3'-*O*-TBDMS phosphorothioate triester using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich).

A solution of the dimer 5'-OH-T-dA^{Bz}-3'-*O*-TBDMS phosphorothioate triester (223 mg, 0.26 mmol) and 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl phosphoramidite (432 mg, 0.50 mmol, 1.9 eq) in anhydrous acetonitrile (20 ml) is added to poly(4-vinylpyridinum *p*-toluenesulfonate) (0.8 g, 2.7 mmol tos$^-$, 10.3 eq). The resulting mixture is shaken for 6 h 30. The reaction is followed by ^{31}P NMR and the yield is also determined by ^{31}P NMR. The desired trimer 5'-*O*-DMTr-dA^{Bz}-T-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphite triester is obtained with 62% of yield compared to the dimer 5'-OH-T-dA^{Bz}-3'-*O*-TBDMS phosphorothioate triester. The crude is a mixture of 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl phosphoramidite (^{31}P NMR (CD_3CN) d 149.14, 8.4%), 5'-*O*-DMTr-dA^{Bz}-T-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphite triester δ(140.90, 140.77, 67.85, 67.79, 43.3%), 5'-OH-T-dA^{Bz}-3'-*O*-TBDMS phosphorothioate triester (δ 68.03, 67.89, 13.4%), 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl hydrogenophosphonate (δ 8.71,8.66, 34.9%).

Sulfurization: The poly(4-vinylpyridinum *p*-toluenesulfonate) is filtered off and the resulting solution is added to AMBERLYST A26 tetrathionate form (0.78 g, 1.33 mmol $S_4O_6^{2-}$, 5 eq.). The reaction is followed by ^{31}P NMR and the yield is also determined by ^{31}P NMR. After 14 h 30 the desired trimer 5'-*O*-DMTr-dA^{Bz}-T-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphorothioate triester is obtained with 100% of yield. The crude is a mixture of 5'-*O*-DMTr-dA^{Bz}-3'- cyanoethyl thiophosphoramidate (^{31}P NMR (CD_3CN) d 71.88, 71.21, 10%), 5'-*O*-DMTr-dA^{Bz}-T-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphorothioate triester (25.9%) and 5'-OH-T-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphorothioate triester (16.2%)

(δ 68.08, 68.05, 67.93, 67.89, 67.85, 67.79, 67.57), 5'-*O*-DMTr-T-3'-cyanoethyl phosphorothioate diester (δ 57.38, 4.8%), 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl hydrogenophosphonate (δ 8.75,8.70, 43.11%). MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1631.68, m/z_{calc} = 1632.77; (negative mode, trihydroxyacetophenone as matrix) ammonia treatment of an aliquot gives 5'-OH-T-dA- 3'-*O*-TBDMS phosphorothioate diester: $[M-H]^-$ m/z_{exp} = 1316.45, m/z_{calc} = 1316.43.

Example 7

Synthesis of the dimer 5'-O-DMTr-dC^{Bz}-T-3'-O-Lev cyanoethyl phosphite triester.

Coupling procedure of 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl phosphoramidite with 5'-OH-T-3'-*O*-Lev using the DOWEX 50W X8 pyridinium form.

Analytical scale.

5'-OH-T-3'-*O*-Lev (20 mg, 58.9 mmol) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl phosphoramidite (83.4 mg, 100 mmol, 1.7 eq) are dissolved in anhydrous acetonitrile (550 ml). The solution is transferred under argon in a NMR tube containing the DOWEX 50W X8 pyridinium form (181 mg, 0.54 mmol $pyrH^+$, 9.2 eq). The reaction is followed by ^{31}P NMR. Before the NMR experiment deuterated acetonitrile (50 ml) is added. The yield is determined by ^{31}P NMR. After 6 h the desired dimer T-dC^{Bz} phosphite triester is obtained with 100% of yield compared to 5'-OH-T-3'-*O*-Lev. The crude is a mixture of 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl phosphoramidite (^{31}P NMR (CD_3CN) δ 149.36,149.32, 11%), 5'-*O*-DMTr-T-dC^{Bz}-3'-*O*-TBDMS cyanoethyl phosphite triester (δ 140.52, 140.39, 70%), 5'-*O*-DMTr- dC^{Bz} -3'-cyanoethyl hydrogenophosphonate (δ 8.90, 8.58, 19%).

Example 8

Synthesis of the dimer 5'-OH-dC^{Bz}-T-3'-O-Lev cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl phosphoramidite with 5'-OH-T-3'-*O*-Lev using the DOWEX 50W X8 pyridinium form.

A solution of 5'-OH-T-3'-*O*-Lev (119 mg, 0.35 mmol) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl phosphoramidite (496 mg, 0.60 mmol, 1.7 eq) in anhydrous acetonitrile (10 ml) is added to DOWEX 50W X8 pyridinium form (1,1 g, 3,3 mmol $pyrH^+$, 9.4 eq). The resulting mixture is shaken for 5 h 30 min. The reaction is

followed by ^{31}P NMR and the yield is also determined by ^{31}P NMR. The desired dimer 5'-*O*-DMTr-dC^{Bz}-T-3'-*O*-Lev cyanoethyl phosphite triester is obtained with 100% of yield compared to 5'-OH-T-3'-*O*-Lev. The crude is a mixture of 5'-*O*-DMTr-T-dC^{Bz}-3'-*O*-Lev cyanoethyl phosphite triester (^{31}P NMR (CD_3CN) δ 140.59, 140.45, 64%), 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl hydrogenophosphonate (δ 8.68, 8.66, 36%).

Sulfurization: The DOWEX 50W X8 resin is filtered off and the resulting solution is added to AMBERLYST A26 tetrathionate form (1.44 g, 2.44 mmol $S_4O_6^{2-}$, 7 eq.). The reaction is followed by ^{31}P NMR. The reaction mixture is shaken for 16 h. The desired dimer 5'-*O*-DMTr-dC^{Bz}-T-3'-*O*-Lev cyanoethyl phosphorothioate triester is isolated after filtration of the resin, evaporation of the solvent and column chromatography (silica gel; CH_2Cl_2 / MeOH (97/3)). The crude is a mixture of 5'-*O*-DMTr-T-dC^{Bz}-3'-*O*-Lev cyanoethyl phosphorothioate triester (^{31}P NMR (CD_3CN) δ 68.05, 67.89, 83.7%), 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl hydrogenophosphonate (δ 8.63, 16.3%). The spectrophotometric purity determined by HPLC at 260 nm is 80%.

Detrytilation of the dimer 5'-*O*-DMTr-dC^{Bz}-T-3'-*O*-Lev cyanoethyl phosphorothioate triester with the DOWEX 50 W X8 H^+ form (Aldrich).

To the mixture of the dimer 5'-*O*-DMTr-dC^{Bz}-T-3'-*O*-Lev cyanoethyl phosphorothioate triester (121 mmol estimated) and 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate diester (44 mmol estimated) in solution in 10 ml of CH_2Cl_2/MeOH (7/3) is added the DOWEX 50 W X8 H^+ form (1.4 g, 7 mmol H^+, 58 eq / dimer). The reaction is followed by reverse phase HPLC. After 15 min the detritylation is complete. The resin is filtered off and the solvents are evaporated. The desired dimer 5'-OH-dC^{Bz}-T-3'-*O*-Lev cyanoethyl phosphorothioate triester is purified by precipitation from CH_2Cl_2/MeOH (9/1) in diethylether. ^{31}P NMR (CD_3OD) δ 68.24, 67.90, MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 803.11 m/z_{calc} = 803.76. The spectrophotometric purity (97 %) is determined by HPLC at 260 nm.

Example 9

Synthesis of the dimer 5'-OH-T-T-3'-O-Lev cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite with 5'-OH-T-3'-*O*-Lev using the DOWEX 50W X8 pyridinium form.

A solution of 5'-OH-T-3'-*O*-Lev (100 mg, 0.29 mmol) and 5'-*O*-DMTr-T-3'-

cyanoethyl phosphoramidite (547 mg, 0.73 mmol, 2.5 eq) in anhydrous acetonitrile (10 ml) is added to DOWEX 50W X8 pyridinium form (0.9 g, 2.7 mmol $pyrH^+$, 9.3 eq). The resulting mixture is shaken for 10 h. The reaction is followed by ^{31}P NMR and by reverse phase HPLC. The excess of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite is hydrolysed with 500 ml of water The desired dimer 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphite triester is obtained with 100% of yield compared to 5'-OH-T-3'-*O*-Lev. The crude is a mixture of 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphite triester (HPLC % Area = 55%) and 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (HPLC % Area = 45%) .

Sulfurization: The DOWEX 50W X8 resin is filtered off and the resulting solution is added to AMBERLYST A26 tetrathionate form (0.8 g, 1.5 mmol $S_4O_6^{2-}$, 5 eq.). The reaction is followed by ^{31}P NMR and by reverse phase HPLC. After 15 h the desired dimer 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester is obtained with 100% of yield. The crude is a mixture of 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester (^{31}P NMR d 68.04, HPLC % Area = 57%), 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (d 8.77, HPLC % Area = 43%).

Detrytilation of the dimer 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester with the DOWEX 50 W X8 H^+ form (Aldrich).

The AMBERLYST A26 resin is filtered off and the solvents are evaporated. To the mixture of the dimer 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester (0.29 mmol estimated) and 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (0.22 mmol estimated) in solution in 20 ml of CH_2Cl_2/MeOH (7/3) is added the DOWEX 50 W X8 H^+ form (3.7 g, 18.5 mmol H^+, 64 eq / dimer). The reaction is followed by reverse phase HPLC. After 30 min the detritylation of the dimer is complete. The resin is filtered off and the solvents are evaporated. The desired dimer 5'-OH-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester is purified by precipitation from CH_2Cl_2/MeOH (9/1) in diethylether. ^{31}P NMR (CD_3CN) d 67.88, 67.73. MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 713.79 m/z_{calc} = 714.66. The purity (95%) is determined by HPLC.

Example 10

Synthesis of dimer 5'-O-DMTr-dA^{Bz}-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate triester dimer.

Coupling procedure of 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl-phosphoramidite with

5'-OH-dA^{Bz}-3'-*O*-TBDMS using the DOWEX 50W X8 pyridinium form:

5'-OH-dA^{Bz}-3'-*O*-TBDMS (100 mg, 0.21 mmol) and 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl-phosphoramidite (311 mg, 0.36 mmol, 1.7 eq) are dissolved in anhydrous acetonitrile (15 ml). The solution is transferred under argon in a flask containing the DOWEX 50W X8 pyridinium form (655 mg, 1.97 mmol pyrH$^+$, 9.2 eq) and is shaken for 4 h 30 min. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. The desired dA-dA phosphite triester dimer is obtained with 92% of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-TBDMS. The crude is a mixture of 5'-O-DMTr-dA^{Bz}-3'-cyanoethyl phosphoramidite (^{31}P NMR (CD_3CN) δ 149.25, 149.13; 27.7%), 5'-O-DMTr-dA^{Bz}-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphite triester (δ 140.75, 140.38; 53.9%), 5'-O-DMTr-dA^{Bz}-3'-cyanoethyl hydrogenophosphonate (δ 8.69, 8.64; 18.5%).

Sulfurization: To a solution of 5'-*O*-DMTr-dA^{Bz}-dA^{Bz}-3'-*O*-TBDMS phosphite triester dimer (0.2 mmol) in anhydrous acetonitrile is added AMBERLYST A26 tetrathionate form (5.4 eq., 1.14 mmol $S_4O_6^{2-}$, 0.63 g). The reaction mixture is shaken for 20 h. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. After filtration of the resins the desired dimer dA-dA phosphorothioate triester is obtained with 88% of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-TBDMS. The crude is a mixture of 5'-O-DMTr-dA^{Bz}-3'-cyanoethyl thiophosphoramidate (^{31}P NMR (CD_3CN) δ 71.85, 71.22; 29.0%), 5'-O-DMTr-dA^{Bz}-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate (δ 68.08, 68.01; 51.5%), 5'-O-DMTr-dA^{Bz}-3'-cyanoethyl hydrogenophosphonate (δ 8.66, 8.59; 19.5%).

Example 11

Synthesis of the dimer 5'-OH-dC^{Bz}-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphoramidite with 5'-OH-dA^{Bz}-3'-*O*-TBDMS using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-dA^{Bz}-3'-*O*-TBDMS adenosine (100 mg, 0.21 mmol) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphoramidite (365 mg, 0.43 mmol, 2. eq) are dissolved in anhydrous acetonitrile (15 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (655 mg, 2.17 mmol tos$^-$, 10.2 eq) and is shaken for 5 h 50 min. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. The desired dC-dA phosphite triester dimer is obtained with 100% of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-

TBDMS. The crude is a mixture of 5'-O-DMTr-dC^{Bz}-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphite triester (^{31}P NMR (CD_3CN) (δ 140.55, 140.49; 53.9%), 5'-O-DMTr-dC^{Bz}-3'-cyanoethyl hydrogenophosphonate (δ 8.67; 18.5%).

Sulfurization: To a solution of 5'-*O*-DMTr-dC^{Bz}-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphite triester dimer (0.21 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (0.63 g, 1.14 mmol $S_4O_6^{2-}$, 5.3 eq.). The reaction mixture is shaken for 14 h 30 min. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. After filtration of the resins the desired dC-dA phosphorothioate triester dimer is obtained with 100% of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-TBDMS. The crude is a mixture of 5'-O-DMTr-dC^{Bz}-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate (^{31}P NMR (CD_3CN) (d 68.14, 68.07; 50.6%), 5'-O-DMTr-dC^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.67; 49.4%). Purification is attempted on a silica gel column, which is treated with triethylamine. Chromatography leads to complete loss of the cyanoethyl group. The dC^{Bz}-dA^{Bz} phosphorothioate dimer is eluted with CH_2Cl_2/CH_3OH (80/1). The appropriates fractions are collected and evaporated to give a colorless oil. Yield: 185 mg, 0.14 mmol, 68%; ^{31}P NMR (CD_3CN) d 57.58, 57.45; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M\text{-}DMTr+2H]^+$ m/z_{exp} = 879.42, m/z_{calc} = 878.97.

Example 12

Synthesis of the dimer 5'-OH-dA^{Bz}-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl-phosphoramidite with 5'-OH-dA^{Bz}-3'-*O*-TBDMS using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-dA^{Bz}-3'-*O*-TBDMS (102 mg, 0.22 mmol) and 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl-phosphoramidite (381 mg, 0.44 mmol, 2.05 eq) are dissolved in anhydrous dichloromethane (15 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (655 mg, 2.19 mmol tos$^-$, 10.1 eq) and is shaken for 5 h 40 min. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. The desired dA-dA phosphite triester dimer is obtained with 100% of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-TBDMS. The crude is a mixture of 5'-O-DMTr-dA^{Bz}-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphite triester (^{31}P NMR (CD_3CN) (d 140.77, 140.46; 66.9%), 5'-O-DMTr-dA^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.50, 8.41; 33.1%).

Sulfurization: To a solution of 5'-*O*-DMTr-dA^{Bz}-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphite triester dimer (0.22 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (0.87 g, 1.14 mmol $S_4O_6^{2-}$, 5.4 eq.). The reaction mixture is shaken for 22 h. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. After filtration of the resins the desired dA-dA phosphorothioate triester dimer is obtained with 100 % of yield compared to the 5'-OH-A^{Bz}-3'-*O*-TBDMS. The crude is a mixture of 5'-O-DMTr-dA^{Bz}-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate (^{31}P NMR (CD_3CN) (d 68.17, 67.89; 62.3%), 5'-O-DMTr-dA^{Bz}-3'-cyanoethyl hydrogenophosphonate (δ 8.45, 8.35; 37.7%).

Detritylation: To a solution of 5'-*O*-DMTr-dA^{Bz}-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphorothioate triester (0.22 mmol) in 10 ml CH_2Cl_2/CH_3OH (7/3) is added 0.63 ml (0.3 mmol, 1.4 eq.) of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 45 min at 0°C. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried (Na_2SO_4), evaporated, and purified on a silica gel column. The desired dA-dA dimer is eluted with CH_2Cl_2/CH_3OH (33/1). The appropriates fractions are collected and evaporated to give a colorless oil. Yield: 73 mg, 76 mmol, 35%; ^{31}P NMR (CD_3CN) δ 67.80, 67.71; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 957.01, m/z_{calc} = 957.07; HPLC (spectrophotometrical purity at 260 nm = 95 %).

Example 13

Synthesis of the dimer 5'-OH-dG^{iBu}-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphoramidite with 5'-OH-dA^{Bz}-3'-*O*-TBDMS using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-dA-3'-*O*-TBDMS (100 mg, 0.21 mmol) and 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphoramidite (352 mg, 0.42 mmol, 1.97 eq) are dissolved in anhydrous acetonitrile (20 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (655 mg, 2.19 mmol tos$^-$, 10.3 eq) and is shaken for 5 h 30 min. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. The desired dG-dA phosphite triester dimer is obtained with 100% of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-TBDMS. The crude is a mixture of 5'-O-DMTr-dG^{iBu}-dA^{Bz}-3'-O-TBDMS cyano-

ethyl phosphite triester (^{31}P NMR (CD_3CN) (δ 140.65, 140.45; 51.2%), 5'-O-DMTr-dG^{iBu}-3'-cyanoethyl hydrogenophosphonate (δ 9.00, 8.81; 48.8%).

Sulfurization: To a solution of 5'-*O*-DMTr-dG^{iBu}-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphite triester dimer (0.21 mmol) in anhydrous acetonitrile is added AMBERLYST A26 tetrathionate form (0.63 g, 1.14 mmol $S_4O_6^{2-}$, 5.4 eq.). The reaction mixture is shaken for 2 h. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. After filtration of the resins the desired dG-dA phosphorothioate triester dimer is obtained with 100% of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-TBDMS. The crude is a mixture of 5'-O-DMTr-dG^{iBu}-dA^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate (^{31}P NMR (CD_3CN) (δ 68.15, 68.02; 50.4%), 5'-O-DMTr-dG^{iBu}-3'-cyanoethyl hydrogenophosphonate (δ 8.91, 8.68; 49.6%).

Detritylation: To a solution of 5'-*O*-DMTr-dG^{iBu}-dA^{Bz}-3'-*O*-TBDMS cyanoethyl phosphorothioate triester (0.21 mmol) in 10 ml CH_2Cl_2/CH_3OH (7/3) is added 0.5 ml (0.3 mmol, 1.4 eq.) of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 20 min at 0°C. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried (Na_2SO_4), evaporated, and purified on a silica gel column. The desired G-A dimer is eluted with CH_2Cl_2/CH_3OH (33/1). The appropriates fractions are collected and evaporated to give a white foam. Yield: 95 mg, 0.1 mmol, 48% with respect of 5'-OH-dA-3'-*O*-TBDM; ^{31}P NMR (CD_3CN) δ 68.10, 67.87; HPLC (spectrophotometrical purity at 260 nm = 80%).

Example 14

Synthesis of the dimer 5'-OH-dG^{iBu}-dC^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphoramidite with 5'-OH-dC^{Bz}-3'-*O*-TBDMS using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-dC^{Bz}-3'-*O*-TBDMS (100 mg, 0.22 mmol) and 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl- phosphoramidite (371 mg, 0.45 mmol, 2 eq) are dissolved in anhydrous acetonitrile (15 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (690 mg, 2.3 mmol tos$^-$, 10.3 eq) and is shaken for 5 h. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. The desired dG-dC phosphite triester dimer is obtained with 100% of yield compared to the 5'-OH-dC^{Bz}-3'-*O*-TBDMS. The

crude is a mixture of 5'-O-DMTr-dG^{iBu}-dC^{Bz}-3'-O-TBDMS cyanoethyl phosphite triester (^{31}P NMR (CD_3CN) (δ 141.73, 141.26; 62.1%), 5'-O-DMTr-dG^{iBu}-3'-cyanoethyl hydrogeno-phosphonate (δ 9.05, 8.88; 37.9%).

Sulfurization: To a solution of 5'-*O*-DMTr-dG^{iBu}-dC^{Bz}-3'-*O*-TBDMS cyanoethyl phosphite triester dimer (0.22 mmol) in anhydrous acetonitrile is added AMBERLYST A26 tetrathionate form (0.65 g, 1.3 mmol $S_4O_6^{2-}$, 5.4 eq.). The reaction mixture is shaken for 2 h. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. After filtration of the resins the desired dG-dC phosphorothioate triester dimer is obtained with 100% of yield compared to the 5'-OH-dC^{Bz}-3'-*O*-TBDMS. The crude is a mixture of 5'-O-DMTr-dG^{iBu}-dC^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate (^{31}P NMR (CD_3CN) (δ 68.12, 67.73; 61.2%), 5'-O-DMTr-dG^{iBu}-3'-cyanoethyl phosphorothioate diester (δ 56.51, 56.39; 25.4%), 5'-O-DMTr-dG^{iBu}-3'-cyanoethyl hydrogenophosphonate (d 9.04, 8.85; 25.4%). ^{31}P NMR d.

Detritytlation: To a solution of 5'-*O*-DMTr-dG^{iBu}-dC^{Bz}-3'-*O*-TBDMS cyanoethyl phosphorothioate triester (0.22 mmol) in 10 ml CH_2Cl_2/CH_3OH (7/3) is added 0.5 ml (0.3 mmol, 1.4 eq.) of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 1 h at 0°C. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried (Na_2SO_4) and evaporated. The crude product is purified on a silica gel column using CH_2Cl_2/CH_3OH (33:1). The appropriate fractions are collected and evaporated to give a colorless oil. Yield: 99 mg, 0.1 mmol, 47%; ^{31}P NMR (CD_3CN) δ 67.82, 67.56; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 914.78, m/z_{calc} = 914.03; HPLC (spectrophotometrical purity at 260 nm = 84%).

Example 15

Synthesis of 5'-O-DMTr-T-T-3'-O-DMTr-phosphorothioate diester.

To a solution of 5'-*O*-DMTr-T-T-3'-*O*-DMTr *H*-phosphonate diester (25 mg, 22 mmol) in dichloromethane is added AMBERLYST A26 tetrathionate form (170 mg, 0.29 mmol $S_4O_6^{2-}$, 13 eq.) and 0.1 mL triethylamine. The reaction mixture is shaken for 78 h. The title compound was isolated after filtration of the resin and evaporation of the solvent. Yield: 28 mg, 22 mmol, 100%; ^{31}P NMR (CD_3CN) d 57.22; MALDI-TOF MS (negative mode, trihydroxy-acetophenone as matrix) $[M-H]^-$ m/z_{exp} = 1166.23, m/z_{calc} = 1666.25.

Example 16

Synthesis of 5'-O-DMTr-T-T-3'-O-TBDMS-phosphorothioate diester.

To a solution of 5'-*O*-DMTr-T-T-3'-*O*-TBDMS *H*-phosphonate (55 mg, 58 mmol) in dichloromethane is added AMBERLYST A26 tetrathionate form (250 mg, 0.42 mmol $S_4O_6^{2-}$, 7.3 eq.) and 0.2 mL triethylamine. The reaction mixture is shaken for 26 h. The title compound was isolated after filtration of the resin and evaporation of the solvent. Yield: 63 mg, 58 mmol, 100%; ^{31}P NMR (CD_3CN) δ 57.78, 57.72; MALDI-TOF MS (negative mode, trihydroxyacetophenone as matrix) $[M-H]^-$ m/z_{exp} = 977.04, m/z_{calc} = 977.13.

Example 17

Synthesis of AMBERLYST A26 tetrathionate form.

10 g commercial Amberlyst A26 hydroxide form (Rohm & Haas) is washed twice with 20 mL methanol and twice with 20 mL dichloromethane and dried in vacuum. Potassium tetrathionate (30.35 g, 100 mmol, 3 eq.) is dissolved in 200 mL deionized water. The solution is added to the resin and shaken for 20 hours. The solution is decanted of. The resin is washed with 4 L deionized water, twice with 100 mL methanol and twice with 100 mL dichloromethane and dried under reduced pressure for 3 hours to give 8.5 g of solid-supported tetrathionate. The reagents loading was determined by elemental analysis, giving a value of 23.25% for sulfur (4.24% for nitrogen, 45.74% for carbon and less than 100 ppm for potassium). Loading: 1.81 mmol $S_4O_6^{2-}$ per gram of resin.

Example 18

Synthesis of polymer-supported pyridinium

The commercially available strongly acidic ion-exchange resin DOWEX 50W X8 H^+ form (Fluka) is washed successively with water, HCl 2M, water until pH 7, methanol and dichloromethane to dry the resin. Then, the resin is stirred in a solution of pyridine 2M in acetonitrile or just washed with a slight flow of the solution of pyridine 2M in acetonitrile for 15 minutes. Then, the resin is washed with acetonitrile and dichloromethane and dried under vacuum over P_2O_5. The reagents loading was determined by elemental analysis, giving a value of 11.56% for sulfur and 3.97% for nitrogen. Loading: 2.83 mmol $pyrH^+$ per gram of resin.

Example 19

Preparation of polystyrene-bound acid chloride

The commercial polystyrene-bound carboxy acid RAPP Polymere (5.0 g, 1.96 mmol/g, 100-200 mesh, 1% DVB) is suspended in anhydrous CH_2Cl_2 (80 ml) and N,N-dimethylformamide (0.3 ml). Thionyl chloride (1.8 ml, 3.5 eq) are added under stirring and the mixture is refluxed for 3h. The resin is filtered under argon and washed with dried CH_2Cl_2 (100 ml), ether (100 ml) and dried under vacuum for 4h.

IR (cm^{-1}): 1775 (C=O, Acid chloride)

Elemental analysis: Cl 7.43% (w/100g resin) (2.09 mmol/g)

Chloride titration: 2.1 mmol/g

Example 20

Synthesis of 5'-O-DMTr-dA^{Bz}-dC^{Bz}-3'-O-Lev H-phosphonate

A solution of 5'-*O*-DMTr-dA^{Bz}-*H*-phosphonate TEA salt (123.4 mg, 0.150 mmol) and of 3'-*O*-Lev-dC^{Bz} (53.7 mg, 0.125 mmol) in 2.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (388.8 mg, 2.1 mmol/g, 5.5 eq) that is suspended in 2.5 ml of the same solvent. The mixture is shaken for 1h at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, dried over Na_2SO_4, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product was dried under vacuum. Yield 89%.

^{31}P NMR (CD_3CN) δ 10.03 ppm, 9.46 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1134.13, m/z_{calc} = 1133.51.

The spectrophotometrical purity determined by HPLC is 93%.

Example 21

Synthesis of 5'-O-DMTr-dA^{Bz}-T-3'-O-Lev H-phosphonate

A solution of 5'-*O*-DMTr-dA^{Bz}-*H*-phosphonate TEA salt (123.4 mg, 0.150 mmol) and of 3'-*O*-Lev-T (42.5 mg, 0.125 mmol) in 2.0 ml of CH_2Cl_2/py (1:1) is

added to polystyrene-bound acid chloride (550.0 mg, 2.1 mmol/g, 7.7 eq) that is suspended in 5.0 ml of the same solvent. The mixture is shaken for 30 min at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, dried over Na_2SO_4, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 88.5%

^{31}P NMR (CD_3CN) δ 10.02 ppm, 9.08 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1043.02, m/z_{calc} = 1045.00.

The spectrophotometrical purity determined by HPLC is 98%.

Example 22

Synthesis of 5'-O-DMTr-T-dC^{Bz} -3'-O-TBDMS H-phosphonate

A solution of 5'-*O*-DMTr-T-*H*-phosphonate TEA salt (106.4 mg, 0.150 mmol) and of 3'-*O*-TBDMS-dC^{Bz} (55.7 mg, 0.125 mmol) in 2.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (555.0 mg, 2.7 mmol/g, 10 eq) that is suspended in 5.0 ml of the same solvent. The mixture is shaken for 2h at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, dried over Na_2SO_4, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 77.5%.

^{31}P NMR (CD_3CN) δ 10.50 ppm, 10.00 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1038.69, m/z_{calc} = 1037.20.

The spectrophotometrical purity determined by HPLC is 94%.

Example 23

Synthesis of 5'-O-DMTr -T-dC^{Bz}-3'-O-TBDMS phosphorothioate TEA salt

A solution of 5'-*O*-DMTr -T-dC^{Bz}-3'-*O*-TBDMS *H*-phosphonate (50 mg, 0.048

mmol) in 5.0 ml of CH_2Cl_2 and 0.2 ml TEA is added to Amberlyst A26 tetrathionate form (141.0 mg, 1.7 mmol/g, 5 eq). The mixture is shaken over night, the the resin is filtered and the solvent is evaporated. The product is dried under vacuum. Yield 100%.

^{31}P NMR (CD_2Cl_2) δ 59.17 ppm, 58.99 ppm.

The spectrophotometrical purity determined by HPLC is 95%.

Example 24

Synthesis of 5'-O-DMTr-dA^{Bz}-T-3'-O-Lev phosphate TEA salt

A solution of 5'-*O*-DMTr-dA^{Bz}-T-3'-*O*-Lev *H*-phosphonate (90 mg, 0.0863 mmol) in 5.0 ml of CH_2Cl_2 and 0.2 ml TEA is added to (polystyrilmethyl)trimethylamonium metaperiodate (NOVABIOCHEM) (173.0 mg, 2.5 mmol/g, 5 eq). The mixture is shaken over night, the resin is filtered and the solvent is evaporated. The product is dried under vacuum. Yield 100%.

^{31}P NMR (CD_2Cl_2) δ −1.37 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1059.31, m/z_{calc} = 1060.03.

The spectrophotometrical purity determined by HPLC is 87%.

Example 25

Synthesis of 5'-O-DMTr-T-dA^{Bz}-dC^{Bz}-3'-O-Lev H-phosphonate

Detritylation of 5'-*O*-DMTr-dA^{Bz}-dC^{Bz}-3'-*O*-Lev *H*-phosphonate

The *H*-phosphonate dimer 5'-*O*-DMTr-dA^{Bz}-dC^{Bz}-3'-*O*-Lev (120 mg, 0,106 mmol) is dissolved in 4.0 ml of CH_2Cl_2/MeOH (7:3) and cooled in an ice bath. To this solution 1.0 ml of a solution of 10% BSA (benzene sulfonic acid) in CH_2Cl_2/MeOH (7:3) is added drop wise under stirring and the progress of the reaction is monitored by TLC. After 15 min the mixture is quenched with a solution of $NaHCO_3$. The organic layer is washed with water to remove any trace of base, then it is dried over Na_2SO_4 and the solvent is evaporated. The product is purified by precipitation from CH_2Cl_2 with ether and dried under vacuum. Yield 88%.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 830.75, m/z_{calc} = 831.75.

The spectrophotometrical purity determined by HPLC is 91%.

Coupling

A solution of 5'-*O*-DMTr-T-*H*-phosphonate TEA salt (93.8 mg, 0.132 mmol) and of 5'-OH-dA^{Bz}-dC^{Bz}-3'-*O*-Lev *H*-phosphonate (73.2 mg, 0.088 mmol) in 2.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (503.0 mg, 2.1 mmol/g, 8 eq) that is suspended in 4.0 ml of the same solvent. The mixture is shaken for 1h at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, dried over Na_2SO_4, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 82%.

^{31}P NMR (CD_2Cl_2) δ 10.23, 10.09, 9.70, 9.68, 9.52, 9.30, 9.24, 9.19 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1421.06, m/z_{calc} = 1422.33.

The spectrophotometrical purity determined by HPLC is 82%.

Example 26

Synthesis of 5'-O-DMTr-dA^{Bz}-dA^{Bz}-T-3'-O-Lev H-phosphonate

Detritylation of 5'-*O*-DMTr-dA^{Bz}-T-3'-*O*-Lev *H*-phosphonate

The *H*-phosphonate dimer 5'-*O*-DMTr-dA^{Bz}-T-3'-*O*-Lev (105 mg, 0,100 mmol) is dissolved in 4.0 ml of CH_2Cl_2/MeOH (7:3) and cooled in an ice bath. 1.0 ml of a solution of 10% BSA in CH_2Cl_2/MeOH (7:3) is added drop wise under stirring and the progress of the reaction is monitored by TLC. After 15 min the mixture is quenched with a solution of $NaHCO_3$. The organic layer is washed with water to remove any trace of base, then it is dried over Na_2SO_4 and the solvent is evaporated. The product is purified by precipitation from CH_2Cl_2 in ether and dried under vacuum. Yield 70%.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 742.25, m/z_{calc} = 742.66.

The spectrophotometrical purity determined by HPLC is 92%.

Coupling

A solution of 5'-*O*-DMTr-dA^{Bz}-*H*-phosphonate TEA salt (69.8 mg, 0.084 mmol) and of 5'-OH-dA^{Bz}-dT-3'-*O*-Lev *H*-phosphonate (52.2 mg, 0.070 mmol) in 2.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (311.0 mg, 2.1 mmol/g, 7.7 eq) that is suspended in 2.0 ml of the same solvent. The mixture is shaken for 3h at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, dried over Na_2SO_4, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 75%.

^{31}P NMR (CD_2Cl_2) δ 10.09, 9.39, 8.82, 8.76, 8.30, 7.56 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1445.60, m/z_{calc} = 1447.40.

The spectrophotometrical purity determined by HPLC is 91.5%.

Example 27

Synthesis of the dimer 5'-OH-T-dG^{iBu}-3'-O-Lev cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-T-3'-cyanoethyl-phosphoramidite with 5'-OH-dG^{iBu}-3'-*O*-Lev using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):
5'-OH-dG^{iBu}-3'-*O*-Lev (201 mg, 0.46 mmol) and 5'-*O*-DMTr-T-3'-cyanoethyl-phosphoramidite (620 mg, 0.83 mmol, 1.8 eq) are dissolved in anhydrous dichloromethane (10 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (1.38 g, 4.6 mmol tos^-, 10 eq) and is shaken for 3 h. The resin is filtered off. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by ^{31}P NMR. The desired T-dG phosphite triester dimer is obtained with 100% of yield compared to the 5'-OH-dG^{iBu}-3'-*O*-Lev. The crude is a mixture of 5'-*O*-DMTr-T-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphite triester ^{31}P NMR ($CDCl_3$) (d 140.76, 139.97; 60.7%) and 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (d 8.10, 8.03; 39.3%).
Sulfurization: To a solution of 5'-*O*-DMTr-T-dG^{iBu}-3'-*O*-Lev cyanoethyl

phosphite triester dimer (0.46 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (1.28 g, 2.3 mmol $S_4O_6^{2-}$, 5 eq.). The reaction mixture is shaken for 2 h. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by ^{31}P NMR. After filtration of the resin the desired T-dG phosphorothioate triester dimer is obtained with 100% of yield compared to the 5'-OH-dG^{iBu}-3'-*O*-Lev. The crude is a mixture of 5'-*O*-DMTr-T-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphorothioate ^{31}P NMR ($CDCl_3$) (d 67.99, 67.71; 64.7%) and 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (d 8.09, 8.02; 35.3%); MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M\text{-}DMTr]^+$ m/z_{exp} = 809.00, m/z_{calc} = 809.77.

Detritytlation: To a solution of 5'-*O*-DMTr-T-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphorothioate triester (0.46 mmol) in 20 ml CH_2Cl_2/CH_3OH (7/3) is added 2 ml (1.2 mmol, 1.4 eq.) of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 25 min at 0°C. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried ($MgSO_4$) and evaporated. The crude product is dissolved in 3.5 mL dichloromethane and added to 50 mL diethylether at 0°C to give a white precipitate. Yield: 370 mg, 0.46 mmol, 99%; ^{31}P NMR ($CDCl_3$) d 67.77, 67.42; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M\text{-}H]^+$ m/z_{exp} = 910.42, m/z_{calc} = 909.77; HPLC HPLC (7.27 min; Area = 85%).

Example 28

Synthesis of the dimer 5'-OH-dA^{Bz}-dG^{iBu}-3'-O-Lev cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl-phosphoramidite with 5'-OH-dG^{iBu}-3'-*O*-Lev using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-dG^{iBu}-3'-*O*-Lev (200 mg, 0.46 mmol) and 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl-phosphoramidite (713 mg, 0.83 mmol, 1.8 eq) are dissolved in anhydrous dichloromethane (10 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (1.38 g, 4.6 mmol tos^-, 10 eq) and is shaken for 2 h. The resin is filtered off. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. The desired dA^{Bz}-dG phosphite triester dimer (HPLC at 11.34 min; Area = 61%) is obtained with 100% of yield compared to the 5'-OH-dG^{iBu}-3'-*O*-Lev (HPLC at 6.34 min; Area = 0%). The crude is a mixture of 5'-*O*-DMTr-dA^{Bz}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphite triester ^{31}P NMR ($CDCl_3$) (d 140.82,

140.30), 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl phosphoramidite (d 149.90) and 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl hydrogeno-phosphonate (d 8.20, 8.03).

Sulfurization: To a solution of 5'-*O*-DMTr-dA^{Bz}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphite triester dimer (0.46 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (1.28 g, 2.3 mmol $S_4O_6^{2-}$, 5 eq.). The reaction mixture is shaken for 2 h. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. After filtration of the resin the desired dA^{Bz}-dG phosphorothioate triester dimer (HPLC at 11.49 min; Area = 61%) is obtained with 100% of yield compared to the 5'-OH-dG^{iBu}-3'-*O*-Lev. The crude is a mixture of 5'-*O*-DMTr-dA^{Bz}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphorothioate ^{31}P NMR ($CDCl_3$) (d 68.01, 67.93), 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl thiophosphoramidate (d 71.86, 71.54) and 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.20, 8.03); MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 1224.14, m/z_{calc} = 1225.27.

Detritylation: To a solution of 5'-*O*-DMTr-dA^{Bz}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphorothioate triester (0.46 mmol) in 20 ml CH_2Cl_2/CH_3OH (7/3) is added 2 ml (1.2 mmol, 1.4 eq.) of a solution of benzene sulfonic acid 10 % in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 20 min at 0°C. Another 1 ml (0.6 mmol, 0.7 eq.) of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3) is added and the solution is stirred for 40 min. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried ($MgSO_4$) and evaporated. The crude product is dissolved in 4 mL dichloromethane and added to 50 mL diethylether at 0°C to give a white precipitate. Yield: 442 mg, 0.4 mmol, 87%; ^{31}P NMR ($CDCl_3$) d 68.09, 67.78; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 923.03, m/z_{calc} = 922.89; HPLC (8.08 min; Area = 85%).

Example 29

Synthesis of the dimer 5'-OH-dC^{Bz}-dG^{iBu}-3'-O-Lev cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphoramidite with 5'-OH-dG^{iBu}-3'-*O*-Lev using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-dG^{iBu}-3'-*O*-Lev (200 mg, 0.46 mmol) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphoramidite (694 mg, 0.83 mmol, 1.8 eq) are dissolved in anhydrous dichloromethane (10 ml). The solution is transferred under argon in a flask

containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (1.38 g, 4.6 mmol tos^-, 10 eq) and is shaken for 3 h. The resin is filtered off. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. The desired dC-dG phosphite triester dimer (HPLC at 12.01 min; Area = 50 %) is obtained with 100% of yield compared to the 5'-OH-dG^{iBu}-3'-*O*-Lev (HPLC at 6.34 min; Area = 0%). The crude is a mixture of 5'-*O*-DMTr-dC^{Bz}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphite triester ^{31}P NMR ($CDCl_3$) (d 140.67, 140.59), 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl phosphoramidite (d 149.93) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl hydrogeno-phosphonate (d 8.08).

Sulfurization: To a solution of 5'-*O*-DMTr-dC^{Bz}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphite triester dimer (0.46 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (1.37 g, 2.5 mmol $S_4O_6^{2-}$, 5.4 eq.). The reaction mixture is shaken for 3 h. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. After filtration of the resin the desired dC-dG phosphorothioate triester dimer (HPLC at 12.26 min; Area = 59%) is obtained with 100% of yield compared to the 5'-OH-dG^{iBu}-3'-*O*-Lev. The crude is a mixture of 5'-*O*-DMTr-dC^{Bz}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphorothioate ^{31}P NMR ($CDCl_3$) (d 68.02, 67.55), 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl thiophosphoramidate (d 71.93, 71.62) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl hydrogeno-phosphonate (d 8.07).

Detritylation: To a solution of 5'-*O*-DMTr-dC^{Bz}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphorothioate triester (0.46 mmol) in 20 ml CH_2Cl_2/CH_3OH (7/3) is added 2 ml (1.2 mmol, 1.4 eq.) of a solution of benzene sulfonic acid 10 % in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 20 min at 0°C. Another 1 ml (0.6 mmol, 0.7 eq.) of a solution of benzene sulfonic acid 10 % in CH_2Cl_2/CH_3OH (7/3) is added and the solution is stirred for 10 min. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried ($MgSO_4$) and evaporated. The crude product is dissolved in 7 mL dichloromethane and added to 50 mL diethylether at 0°C to give a white precipitate. Yield: 493 mg, 0.33 mmol, 71%; ^{31}P NMR ($CDCl_3$) d 67.38, 66.77; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 898.84, m/z_{calc} = 898.86; HPLC (8.34 min; Area = 60 %).

Example 30

Synthesis of the dimer 5'-OH-dG^{iBu}-dG^{iBu}-3'-O-Lev cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphoramidite with

5'-OH-dG^{iBu}-3'-*O*-Lev using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-dG^{iBu}-3'-*O*-Lev (200 mg, 0.46 mmol) and 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphoramidite (698 mg, 0.84 mmol, 1.8 eq) are dissolved in anhydrous dichloromethane (10 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (1.38 g, 4.6 mmol tos$^-$, 10 eq) and is shaken for 1 h 15 min. The resin is filtered off. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. The desired dG-dG phosphite triester dimer (HPLC at 11.01 min; Area = 65%) is obtained with 100% of yield compared to the 5'-OH-dG^{iBu}-3'-*O*-Lev (HPLC at 6.34 min; Area = 0%). The crude is a mixture of 5'-*O*-DMTr-dG^{iBu}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphite triester ^{31}P NMR ($CDCl_3$) (d 141.91, 140.29) and 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl hydrogenophosphonate (d 8.80, 8.06).

Sulfurization: To a solution of 5'-*O*-DMTr-dG^{iBu}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphite triester dimer (0.46 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (1.28 g, 2.3 mmol $S_4O_6^{2-}$, 5 eq.). The reaction mixture is shaken for 1 h 20 min. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. After filtration of the resin the desired dG-dG phosphorothioate triester dimer (HPLC at 11.52 min; Area = 75%) is obtained with 100 % of yield compared to the 5'-OH-dG^{iBu}-3'-*O*-Lev. The crude is a mixture of 5'-*O*-DMTr-dG^{iBu}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphorothioate ^{31}P NMR ($CDCl_3$) (d 68.61, 67.64) and 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl hydrogenophosphonate (d 8.07); MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M\text{-}DMTr]^+$ m/z_{exp} = 904.14, m/z_{calc} = 904.87.

Detritylation: To a solution of 5'-*O*-DMTr-dG^{iBu}-dG^{iBu}-3'-*O*-Lev cyanoethyl phosphorothioate triester (0.46 mmol) in 20 ml CH_2Cl_2/CH_3OH (7/3) is added 2 ml (1.2 mmol, 1.4 eq.) of a solution of benzene sulfonic acid 10 % in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 25 min at 0°C. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried ($MgSO_4$) and evaporated. The crude product is dissolved in 7.5 mL dichloromethane and added to 50 mL diethylether at 0°C to give a white precipitate. Yield: 450 mg, 0.41 mmol, 88 ; ^{31}P NMR ($CDCl_3$) d 67.85, 67.66; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M\text{-}H]^+$ m/z_{exp} = 905.45, m/z_{calc} = 904.87; HPLC (7.95 min; Area = 82%).

Example 31

Synthesis of the trimer 5'-OH-dA^{Bz}-T-dG^{IBu}-3'-O-Lev cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl-phosphoramidite with 5'-OH-T-dG^{IBu}-3'-*O*-Lev cyanoethyl phosphorothioate triester using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-T-dG^{IBu}-3'-*O*-Lev cyanoethyl phosphorothioate triester (362 mg, 0.4 mmol) and 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl-phosphoramidite (623 mg, 0.73 mmol, 1.8 eq) are dissolved in anhydrous dichloromethane (15 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (1.2 g, 4.0 mmol tos$^-$, 10 eq) and is shaken for 6 h 20 min. Water (50 mL) is added to hydrolyze the remaining phosphoramidite. After 1 h the resin is filtered off. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. The desired dA-T-dG phosphite triester trimer (HPLC at 11.32 min; Area = 66%) is obtained with 100 % of yield compared to the 5'-OH-T-dG^{IBu}-3'-*O*-Lev cyanoethyl phosphorothioate triester (HPLC at 7.27 min; Area = 0%). The crude is a mixture of 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl-phosphite-T-3'-cyanoethyl-thionophosphotriester-dG^{IBu}-3'-*O*-Lev trimer ^{31}P NMR ($CDCl_3$) (d 140.97, 140.79, 140.40, 139.90, 67.89, 67.87, 67.83) and 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.12, 8.03).

Sulfurization: To a solution of 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl-phosphite-T-3'-cyanoethyl-thionophosphotriester-dG^{IBu}-3'-*O*-Lev (0.4 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (1.12 g, 2.0 mmol $S_4O_6^{2-}$, 5 eq.). The reaction mixture is shaken for 14 h. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. After filtration of the resin the desired dA-T-dG phosphorothioate triester trimer (HPLC at 11.63 min; Area = 64 %) is obtained with 100 % of yield compared to the 5'-OH-T-dG^{IBu}-3'-*O*-Lev cyanoethyl phosphorothioate triester. The crude is a mixture of 5'-*O*-DMTr-dA^{Bz}-T-dG^{IBu}-3'-*O*-Lev cyanoethyl phosphorothioate triester ^{31}P NMR ($CDCl_3$) (d 68.05, 67.92, 67.84, 67.68) and 5'-*O*-DMTr-dA^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.16, 7.98); MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M\text{-}DMTr]^+$ m/z_{exp} = 1583.16, m/z_{calc} = 1582.53.

Detritylation: To a solution of 5'-*O*-DMTr-dA^{Bz}-T-dG^{IBu}-3'-*O*-Lev cyanoethyl phosphorothioate triester (0.4 mmol) in 60 ml CH_2Cl_2/CH_3OH (7/3) is added 4

ml (2.5 mmol, 6.3 eq.) of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 30 min at 0°C. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried ($MgSO_4$) and evaporated. The crude product is dissolved in 10 mL dichloromethane and added to 50 mL diethylether at 0°C to give a white precipitate. Yield: 543 mg, 0.32 mmol, 80 %; ^{31}P NMR ($CDCl_3$) d 67.94, 67.79, 67.72, 67.66, 67.58, 67.28, 67.14; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 1296.07, m/z_{calc} = 1296.22; HPLC (9.39 min; Area = 77%).

Example 32

Synthesis of the tetramer 5'-OH-dC^{Bz}-dG^{iBu}-T-T-3'-O-Lev cyanoethyl phosphorothioate triester.

1st Coupling procedure of 5'-*O*-DMTr-T-3'-cyanoethyl-phosphoramidite with 5'-OH-T-3'-*O*-Lev using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich) providing the 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphite triester dimer:

5'-OH-T-3'-*O*-Lev (340 mg, 1.0 mmol) and 5'-*O*-DMTr-T-3'-cyanoethyl-phosphoramidite (1266 mg, 1.7 mmol, 1.7 eq) are dissolved in anhydrous dichloromethane (10 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (3.0 g, 10 mmol tos^-, 10 eq) and is shaken for 5 h 15 min. Water (50 mL) is added to hydrolyze the remaining phosphoramidite. After 1 h 15 min the resin is filtered off. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. The desired T-T phosphite triester dimer (HPLC at 11.14 min; Area = 71%) is obtained with 100% of yield compared to the 5'-OH-T-3'-*O*-Lev (HPLC at 5.49 min; Area = 0%). The crude is a mixture of 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphite triester ^{31}P NMR ($CDCl_3$) (d 140.58, 140.31) and 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (d 8.10, 8.04).

1st Sulfurization: To a solution of 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphite triester dimer (1.0 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (3.89 g, 7.0 mmol $S_4O_6^{2-}$, 7 eq.). The reaction mixture is shaken for 20 h. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. After filtration of the resin the desired T-T phosphorothioate triester dimer (HPLC at 11.44 min; Area = 75%) is obtained with 100% of yield compared to the 5'-OH-T-3'-*O*-Lev. The crude is a mixture of 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phos-

phorothioate ^{31}P NMR ($CDCl_3$) (d 68.20, 68.14) and 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (d 8.11, 8.05).

1st Detritylation: To a solution of 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester (1.0 mmol) in 70 ml CH_2Cl_2/CH_3OH (7/3) is added 4 ml (2.5 mmol, 2.5 eq.) of a solution of benzene sulfonic acid 10 % in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 40 min at 0°C. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried ($MgSO_4$) and evaporated. The crude product is dissolved in 8 mL dichloromethane and added to 50 mL diethylether at 0°C to give a white precipitate. Yield: 764 mg, 0.99 mmol, 99%; ^{31}P NMR ($CDCl_3$) d 67.87, 67.60; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 713.95, m/z_{calc} = 714.66; HPLC (7.70 min; Area = 88%).

2nd Coupling procedure of 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphoramidite with 5'-OH-T-T-3'-*O*-Lev cyanoethyl phosphorothioate dimer using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich) providing the 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphite-T-3'-cyanoethyl-thionophosphotriester-T-3'-*O*-Lev trimer:

5'-OH-T-T-3'-*O*-Lev cyanoethyl phosphorothioate (764 mg, 0.99 mmol) and 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphoramidite (1400 mg, 1.7 mmol, 1.7 eq) are dissolved in anhydrous dichloromethane (20 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (3.0 g, 10 mmol tos^-, 10 eq) and is shaken for 2 h 50 min. Water (50 mL) is added to hydrolyze the remaining phosphoramidite. After 1 h 20 min the resin is filtered off. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. The desired 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphite-T-3'-cyanoethyl-thionophosphotriester-T-3'-*O*-Lev trimer (HPLC at 11.65 min; Area = 72%) is obtained with 100% of yield compared to the 5'-OH-T-T-3'-*O*-Lev cyanoethyl phosphorothioate (HPLC at 7.70 min; Area = 0%). The crude is a mixture of 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphite-T-3'-cyanoethyl-thionophosphotriester-T-3'-*O*-Lev trimer ^{31}P NMR ($CDCl_3$) (d 142.78, 142.67, 141.64, 141.50, 68.47, 68.39, 68.16, 67.93) and 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl hydrogenophosphonate (d 8.59, 8.05).

2nd Sulfurization: To a solution of 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphite-T-3'-cyanoethyl-thionophosphotriester-T-3'-*O*-Lev trimer (0.99 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (2.78 g, 5.0 mmol $S_4O_6^{2-}$, 5 eq.). The reaction mixture is shaken for 18 h 45 min. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is de-

termined by HPLC. After filtration of the resin the desired dG-T-T phosphorothioate triester trimer (HPLC at 11.66 min; Area = 58%) is obtained with 100% of yield compared to the 5'-OH-T-T-3'-*O*-Lev cyanoethyl phosphorothioate. The crude is a mixture of 5'-DMTr-dG^{iBu}-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester trimer ^{31}P NMR ($CDCl_3$) (d 68.94, 68.43, 68.09, 67.93, 67.69, 67.34); MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 1484.81, m/z_{calc} = 1485.47 and 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl hydrogenophosphonate (d 8.59, 8.52).

2nd Detritylation: To a solution of 5'-DMTr-dG^{iBu}-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester trimer (0.99 mmol) in 75 ml CH_2Cl_2/CH_3OH (7/3) is added 4 ml (2.5 mmol, 2.5 eq.) of a solution of benzene sulfonic acid 10 % in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 40 min at 0°C. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried ($MgSO_4$) and evaporated. The crude product is dissolved in 8 mL dichloromethane and added to 50 mL diethylether at 0°C to give a white precipitate. Yield: 1257 mg, 0.78 mmol, 79%; ^{31}P NMR ($CDCl_3$) d 68.32, 68.07, 68.01, 67.95, 67.86, 67.51, 67.25; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 1182.12, m/z_{calc} = 1183.09; HPLC (8.94 min; Area = 79%).

3rd Coupling procedure of 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphoramidite with 5'-OH-dG^{iBu}-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester trimer using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich) providing the 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphite-dG^{iBu}-cyanoethyl-thionophosphotriester-T-3'-cyanoethyl-thionophosphotriester-T-3'-*O*-Lev tetramer:

5'-OH-dG^{iBu}-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester trimer (1257 mg, 0.78 mmol) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphoramidite (1420 mg, 1.7 mmol, 2.2 eq) are dissolved in anhydrous dichloromethane (20 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (3.0 g, 10 mmol tos$^-$, 12.8 eq) and is shaken for 4 h 50 min. Water (100 mL) is added to hydrolyze the remaining phosphoramidite. After 20 min the resin is filtered off. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. The desired 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphite-dG^{iBu}-3'-cyanoethyl-thionophosphotriester-T-3'-cyanoethyl-thionophosphotriester-T-3'-*O*-Lev tetramer (HPLC at 12.04 min; Area = 79%) is obtained with 100% of yield compared to the 5'-OH-dG^{iBu}-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester trimer (HPLC at 8.94 min; Area = 0%). The crude is a mixture of 5'-*O*-DMTr-dC^{Bz}-3'-

cyanoethyl-phosphite-dG^{iBu}-3'-cyanoethyl-thionophosphotriester-T-3'-cyanoethyl-thionophosphotriester-T-3'-*O*-Lev tetramer ^{31}P NMR ($CDCl_3$) (d 141.01, 140.93, 140.05, 139.90, 68.50, 68.09, 68.04, 67.95) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.15).

3rd Sulfurization: To a solution of 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphite-dG^{iBu}-3'-cyanoethyl-thionophosphotriester-T-3'-cyanoethyl-thionophosphotriester-T-3'-*O*-Lev trimer (0.78 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (2.78 g, 5.0 mmol $S_4O_6^{2-}$, 6.4 eq.). The reaction mixture is shaken for 3 h. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. After filtration of the resin the desired dC-dG-T-T phosphorothioate triester tetramer (HPLC at 121.18 min; Area = 73%) is obtained with 100% of yield compared to the 5'-OH-dG^{iBu}-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester trimer. The crude is a mixture of 5'-DMTr-dC^{Bz}-dG^{iBu}-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester tetramer ^{31}P NMR ($CDCl_3$) (d 68.51, 68.45, 68.22, 68.18, 68.10, 68.09, 67.70, 67.65); MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 1947.90, m/z_{calc} = 1947.89 and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.09).

3rd Detritylation: To a solution of 5'-DMTr-dC^{Bz}-dG^{iBu}-T-T-3'-*O*-Lev cyanoethyl phosphorothioate triester tetramer (0.78 mmol) in 80 ml CH_2Cl_2/CH_3OH (7/3) is added 7 ml (4.4 mmol, 5.6 eq.) of a solution of benzene sulfonic acid 10 % in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 50 min at 0°C. The reaction is washed with 10 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried ($MgSO_4$) and evaporated. The crude product is dissolved in 12 mL CH_2Cl_2/CH_3OH (2/1) and added to 100 mL diethylether at 0°C to give a white precipitate. Yield: 1334 mg, 0.53 mmol, 68%; ^{31}P NMR ($CDCl_3$) d 68.50, 68.41, 67.93, 67.86, 67.81, 67.74, 67.71, 67.64, 67.57, 67.51; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 1645.24, m/z_{calc} = 1645.52; HPLC (9.95 min; Area = 68%).

Example 33

Synthesis of the dimer 5'-OH-dC^{Bz}-dC^{Bz}-3'-O-TBDMS cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphoramidite with 5'-OH-dC^{Bz}-3'-*O*-TBDMS using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-dC^{Bz}-3'-*O*-TBDMS (100 mg, 0.22 mmol) and 5'-*O*-DMTr-dC^{Bz}-3'-

cyanoethyl-phosphoramidite (384 mg, 0.46 mmol, 2.1 eq) are dissolved in anhydrous acetonitrile (15 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (690 mg, 2.3 mmol tos^-, 10.3 eq) and is shaken for 6 h. The resin is filtered off. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. The desired dC-dC phosphite triester dimer is obtained with 100% of yield compared to the 5'-OH-dG^{iBu}-3'-*O*-TBDMS. The crude is a mixture of 5'-*O*-DMTr-dC^{Bz}-dC^{Bz}-3'-*O*-TBDMS cyanoethyl phosphite triester ^{31}P NMR ($CDCl_3$) (d 141.06, 140.93) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.67).

Sulfurization: To a solution of 5'-*O*-DMTr-dC^{Bz}-dC^{Bz}-3'-*O*-TBDMS cyanoethyl phosphite triester dimer (0.22 mmol) in anhydrous acetonitrile is added AMBERLYST A26 tetrathionate form (620 mg, 1.2 mmol $S_4O_6^{2-}$, 5.4 eq.). The reaction mixture is shaken for 65 h. The reaction is followed by ^{31}P NMR. The yield is determined by ^{31}P NMR. After filtration of the resin the desired dC-dC phosphorothioate triester dimer is obtained with 100 % of yield compared to the 5'-OH-dC^{Bz}-3'-*O*-TBDMS. The crude is a mixture of 5'-*O*-DMTr-dC^{Bz}-dC^{Bz}-3'-*O*-TBDMS cyanoethyl phosphorothioate ^{31}P NMR ($CDCl_3$) (d 68.24, 68.19) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.57).

Detritytlation: To a solution of 5'-*O*-DMTr-dC^{Bz}-dC^{Bz}-3'-*O*-TBDMS cyanoethyl phosphorothioate triester (0.22 mmol) in 20 ml CH_2Cl_2/CH_3OH (7/3) is added 0.5 ml (0.3 mmol, 1.3 eq.) of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 30 min at 0°C. Another 0.5 ml (0.3 mmol, 1.3 eq.) of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3) is added and the solution is stirred for 30 min. Another 0.7 ml (0.4 mmol, 1.8 eq.) of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3) is added and the solution is stirred for 30 min. The reaction is washed with 5 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried ($NaSO_4$), evaporated and purified on a silica gel column. The desired dimer dC-dC is eluted with CH_2Cl_2/CH_3OH (33/1). Yield: 106 mg, 0.1 mmol, 52%; ^{31}P NMR ($CDCl_3$) d 67.92, 67.78; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 908.47, m/z_{calc} = 909.02; HPLC (12.73 min; Area = 85%).

Example 34

Synthesis of the dimer 5'-OH-dG^{iBu}-dA^{Bz}-3'-O-Lev cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dG^{iBu}-3'-cyanoethyl-phosphoramidite with

5'-OH-dA^{Bz}-3'-*O*-Lev using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-dA^{Bz}-3'-*O*-Lev (2.235 g, 4.93 mmol) and 5'-*O*-DMTr-dG^{IBu}-3'-cyanoethyl-phosphoramidite (6.13 g, 7.43 mmol, 1.5 eq) are dissolved in anhydrous dichloromethane (100 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (14.76 g, 49.3 mmol tos$^-$, 10 eq) and is shaken for 2 h 45 min. Water (0.2 ml) is added and the mixture is shaken for 1 h 25 min. The resin is filtered off. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. The desired dG-dA phosphite triester dimer (HPLC at 11.69 min; Area = 65 %) is obtained with 100% of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-Lev (HPLC at 7.20 min; Area = 0%). The crude is a mixture of 5'-*O*-DMTr-dG^{IBu}-dA^{Bz}-3'-*O*-Lev cyanoethyl phosphite triester ^{31}P NMR ($CDCl_3$) (d 140.52, 140.20) and 5'-*O*-DMTr-dG^{IBu}-3'-cyanoethyl hydrogenophosphonate (d 8.59, 8.09).

Sulfurization: To a solution of 5'-*O*-DMTr-dG^{IBu}-dA^{Bz}-3'-*O*-Lev cyanoethyl phosphite triester dimer (4.93 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (19.7 g, 24.63 mmol $S_4O_6^{2-}$, 5 eq.). The reaction mixture is shaken for 3 h 10 min. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by ^{31}P NMR. After filtration of the resin the desired dG-dA phosphorothioate triester dimer is obtained with 100 % of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-Lev. The crude is a mixture of 5'-*O*-DMTr-dG^{IBu}-dA^{Bz}-3'-*O*-Lev cyanoethyl phosphorothioate ^{31}P NMR ($CDCl_3$) (d 68.45, 67.73) and 5'-*O*-DMTr-dG^{IBu}-3'-cyanoethyl hydrogenophosphonate (d 8.60, 8.04).

Detritylation: To a solution of 5'-*O*-DMTr-dG^{IBu}-dA^{Bz}-3'-*O*-Lev cyanoethyl phosphorothioate triester (4.93 mmol) in 200 ml dichloromethane is added 50 ml methanol and 20 ml (12.6 mmol, 2.6 eq.) of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 40 min at 0°C. Another 6 ml (3.8 mmol, 0.8 eq.) of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3) is added and the solution is stirred for 15 min. Another 4 ml (2.5 mmol, 0.5 eq.) of a solution of benzene sulfonic acid 10 % in CH_2Cl_2/CH_3OH (7/3) is added and the solution is stirred for 15 min. The reaction is washed with 70 ml of a saturated solution of $NaHCO_3$, the organic layer is separated, dried ($MgSO_4$) and evaporated. The crude product is dissolved in 16 mL dichloromethane and added to 100 ml diethylether at 0°C to give a white precipitate. Yield: 4.28 mg, 3.8 mmol, 77%; ^{31}P NMR ($CDCl_3$) d 68.07, 67.80; MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) [M-

H]$^+$ m/z_{exp} = 923.08, m/z_{calc} = 922.89; HPLC (8.90 min and 9.07 min; Area = 70%). After complete deprotection with ammonia solution (28% NH_3 in water, 14 h) MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) [M-H]$^+$ m/z_{exp} = 597.57, m/z_{calc} = 596.51; HPLC (8.45 min and 8.77 min; Area = 82%).

Example 35

Synthesis of the dimer 5'-DMTr-O-dC^{Bz}-dA^{Bz}-3'-OH cyanoethyl phosphorothioate triester.

Coupling procedure of 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphoramidite with 5'-OH-dA^{Bz}-3'-*O*-Lev using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-dA^{Bz}-3'-*O*-Lev (200 mg, 0.44 mmol) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl-phosphoramidite (626 mg, 0.75 mmol, 1.7 eq) are dissolved in anhydrous dichloromethane (10 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (1.3 g, 3 mmol tos$^-$, 6.8 eq) and is shaken for 8 h. The resin is filtered off. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by HPLC. The desired dC-dA phosphite triester dimer (HPLC at 13.13 min; Area = 63%) is obtained with 100% of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-Lev (HPLC at 7.39 min; Area = 0%). The crude is a mixture of 5'-*O*-DMTr-dG^{Bz}-dA^{Bz}-3'-*O*-Lev cyanoethyl phosphite triester ^{31}P NMR ($CDCl_3$) (d 140.68, 140.58) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.04, 8.02).

Sulfurization: To a solution of 5'-*O*-DMTr-dC^{Bz}-dA^{Bz}-3'-*O*-Lev cyanoethyl phosphite triester dimer (0.44 mmol) in anhydrous dichloromethane is added AMBERLYST A26 tetrathionate form (2.5 g, 4.38 mmol $S_4O_6^{2-}$, 9.9 eq.). The reaction mixture is shaken for 10 h. The reaction is followed by reverse phase HPLC and ^{31}P NMR. The yield is determined by ^{31}P NMR. After filtration of the resin the desired dC-dA phosphorothioate triester dimer is obtained with 100% of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-Lev. The Solvent is removed under reduced pressure. The crude is a mixture of 5'-*O*-DMTr-dC^{Bz}-dA^{Bz}-3'-*O*-Lev cyanoethyl phosphorothioate ^{31}P NMR ($CDCl_3$) (d 68.33, 68.30) and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.04, 8.02).

Deprotection of the levulinyl group: The 5'-*O*-DMTr-dC^{Bz}-dA^{Bz}-3'-*O*-Lev cyanoethyl phosphorothioate triester (0.44 mmol) is dissolved in 16 ml pyridine and 4 ml acetic acid. AMBERLYST 15 hydrazine form (1 g, 3.91 mmol $N_2H_5^-$, 8.7 eq.) is added and the solution is shaken for 1 h 30 min. The reaction

is followed by reverse phase HPLC. The yield is determined by reverse phase HPLC. After filtration of the resin the desired dC-dA phosphorothioate triester dimer is obtained with 100 % of yield compared to the 5'-OH-dA^{Bz}-3'-*O*-Lev. The Solvent is removed under reduced pressure. The crude is a mixture of 5'-*O*-DMTr-dC^{Bz}-dA^{Bz}-3'-OH cyanoethyl phosphorothioate HPLC (12.24 min and 12.45 min; Area = 66%)and 5'-*O*-DMTr-dC^{Bz}-3'-cyanoethyl hydrogenophosphonate HPLC (10.71 min and 10.82 min; Area = 34 %). MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M-H]^+$ m/z_{exp} = 1120.23, m/z_{calc} = 1121.16.

SYNTHESIS OF RESINS

Example 36

Synthesis of AMBERLYST A26 periodate form.

10 g commercial Amberlyst A26 hydroxide form (Rohm & Haas) is washed twice with 20 ml methanol and twice with 20 ml dichloromethane and dried in vacuum. Potassium tetrathionate (10 g, 47 mmol, 1.4 eq.) is dissolved in 300 ml deionized water. The solution is added to the resin and shaken for 17 hours. The solution is filtered off. Another time Potassium tetrathionate (10 g, 47 mmol, 1.4 eq.) is dissolved in 200 ml deionized water. The solution is added to the resin and shaken for 6 hours. The solution is filtered off. The resin is washed with 1 l deionized water, twice with 30 ml methanol and twice with 30 ml dichloromethane and dried under reduced pressure for 3 hours to give 8.2 g of solid-supported periodate. The reagents loading was determined by elemental analysis, giving a value of 27.16% for iodine (3.40% for nitrogen and 40.20% for carbon. Loading: 2.14 mmol IO_4^- per gram of resin. The resin can be recycled applying the same protocol. Comparable resins are commercially available.

Example 37

Synthesis of AMBERLYST 15 hydrazine form.

5 g commercial Amberlyst 15 H^+ form (Aldrich) is washed successively with 20 ml hydrochloric acid and with 500 ml deionized water. Hydrazine (4.38 g, 87.5 mmol, 3.8 eq.) is dissolved in 100 ml deionized water. The solution is added to the resin and shaken for 16 hours. The solution is filtered off. The resin is washed successively with 500 ml deionized water, 50 ml methanol and with 50 ml dichloromethane and dried under reduced pressure for 3 hours to give 5.2 g of solid-supported hydrazine. The reagents loading was determined by ele-

mental analysis, giving a value of 10.94 % for nitrogen (12.37% for sulfur and 44.19% for carbon. Loading: 3.91 mmol $N_2H_5^+$ per gram of resin.

Example 38

Recycling of poly(4-vinylpyridinum p-toluenesulfonate)

Used poly(4-vinylpyridinum *p*-toluenesulfonate) (20 g) is washed successively for one hour each with acetonitrile (100 ml), dichloromethane (100 ml) and methanol (100 ml). Then the resin is added to a solution of *p*-toluene sulfonic acid (40 g, 0.23 mol) in methanol (400 ml) and shaken for 21 h. The solution is filtered off and the resin is washed with methanol (150 ml) and dichloromethane (150 ml) and dried at 80°C under vacuum over P_2O_5 for 8 h. The reagents loading was determined by elemental analysis, giving a value of 10.73% for sulfur and 4.90% for nitrogen. Loading: 3.35 mmol tos^- per gram of resin.

SYNTHESIS OF PHOSPHODIESTER OLIGOMERS

FORMATION OF THE PHOSPHATE TRIESTER BRIDGE

In this part, we focus on the formation of natural phosphodiester oligomers. The coupling of the phosphoramidite nucleotide and of the 5'-OH nucleoside was performed with the resin polyvinyl pyridinium *p*-toluene sulfonate (PVP) commercially available from ALDRICH. The oxidation of the phosphite triester linkage was achieved with the resin polystyrylmethyltrimethylamonium metaperiodate (PS-$N(CH_3)_3^+$ IO_4^-) commercially available from NOVABIOCHEM. Then, the detritylation step was performed in solution with BSA. The purification was an extraction in dichloromethane followed by a precipitation in ether.

Example 39

Synthesis of the dimer 5'-OH-T-T-3'-O-Lev cyanoethyl phosphate triester.
Coupling procedure of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite with 5'-OH-T-3'-*O*-Lev using the PVP resin.

A solution of 5'-OH-T-3'-*O*-Lev (170 mg, 0.5 mmol) and 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite (745 mg, 1 mmol, 2 eq) in anhydrous acetonitrile (15 ml) is added to PVP resin (1.5 g, 5 mmol $pyrH^+$, 10 eq). The reaction is followed by reverse phase HPLC. After 5h the reaction is complete. The desired dimer 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphite triester is characterized by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphite triester (d 140.62, 140.48) and of 5'-*O*-DMTr-T-3'-cyanoethyl hy-

drogenophosphonate (d 8.81, 8.76).

Oxidation: The PVP resin is filtered off and the resulting solution is added to PS-$N(CH_3)_3^+$ IO_4^- (1 g, 2.5 mmol IO_4^-, 5 eq.). The reaction is followed by ^{31}P NMR and by reverse phase HPLC. The reaction is complete after 45 min. The desired dimer 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphate triester is characterized by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-T-3'-cyanoethyl phosphate diester (d -2.80), 5'-*O*-DMTr-T-T-3'-*O*-Lev cyanoethyl phosphate triester (d -1.48, -1.63), 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (d 8.69, 8.64).

Detritylation: The PS-$N(CH_3)_3^+$ IO_4^- is filtered off and the solvent are evaporated. The crude is dissolved in 8ml of CH_2Cl_2/CH_3OH (7/3) and cooled in an ice bath. To this solution is added 2 ml of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 45 min at 0°C. The reaction is stopped with 20 ml of a saturated solution of $NaHCO_3$. The aqueous phase is extracted three times with dichloromethane. The organic layer is separated, dried (Na_2SO_4) and evaporated under reduce pressure. The residue is dissolved in 5ml of CH_2Cl_2/MeOH (4/1) and added to 50ml of cooled diethyl ether drop by drop with a strong stirring. The mixture is centrifuged for 45 min and the supernatant is eliminated. The desired 5'-OH-T-T-3'-*O*-Lev cyanoethyl phosphate triester dimer is obtained with a yield of 75.4% (calculated: 91% per step) compared to the 5'-OH-T-3'-*O*-Lev. ^{31}P NMR (CD_3CN) d -1.67, -1.72. MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 700.42, m/z_{calc} = 698.60. The spectrophotometric purity (91%) is determined by HPLC at 260 nm.

Example 40

Synthesis of the trimer 5'-OH-G^{iBu}-T-T-3'-O-Lev cyanoethyl phosphate triester.

Coupling procedure of 5'-*O*-DMTr-G^{iBu}-3'-cyanoethyl phosphoramidite with 5'-OH-T-T-3'-*O*-Lev dimer using the PVP resin.

A solution of 5'-OH-T-T-3'-*O*-Lev (263 mg, 377 mmol) and 5'-*O*-DMTr-G^{iBu}-3'-cyanoethyl phosphoramidite (623 mg, 754 mmol, 2 eq) in anhydrous acetonitrile (15 ml) is added to PVP resin (1.1 g, 3.8 mmol $pyrH^+$, 10 eq). The reaction is followed by reverse phase HPLC. After 3h 30min the reaction is complete. The desired trimer 5'-*O*-DMTr-G^{iBu}-T-T-3'-*O*-Lev is characterized by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-G^{iBu}-T-T-3'-*O*-Lev trimer (phosphite triester linkage d 141.56, 141.50, 141.44, 141.39, 141.13, 141.05 ; phosphate

triester linkage d -1.35, -1.44, -1.52, -1.57) and of 5'-*O*-DMTr-G^{IBu}-3'-cyanoethyl hydrogenophosphonate (d 9.03, 8.84).

Oxidation: The PVP resin is filtered off and the resulting solution is added to PS-$N(CH_3)_3^+$ IO_4^- (0.75 g, 1.9 mmol IO_4^-, 5 eq.). The reaction is followed by ^{31}P NMR and by reverse phase HPLC. The reaction is complete after 45 min. The desired dimer 5'-*O*-DMTr-G^{IBu}-T-T-3'-*O*-Lev cyanoethyl phosphate triester is characterized by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-G^{IBu}-3'- cyanoethyl phosphate diester (d -2.52), 5'-*O*-DMTr-G^{IBu}-T-T-3'-*O*-Lev cyanoethyl phosphate triester (d -1.23, -1.35, -1.43, -1.50, -1.55), 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (d 9.09, 8.90).

Detritylation: The PS-$N(CH_3)_3^+$ IO_4^- is filtered off and the solvent are evaporated. The crude is dissolved in 8ml of CH_2Cl_2/CH_3OH (7/3) and cooled in an ice bath. To this solution is added 2 ml of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 45 min at 0°C. The reaction is stopped with 20 ml of a saturated solution of $NaHCO_3$. The aqueous phase is extracted three times with dichloromethane. The organic layer is separated, dried (Na_2SO_4) and evaporated under reduce pressure. The residue is dissolved in 5ml of CH_2Cl_2/MeOH (4/1) and added to 50ml of cooled diethyl ether drop by drop with a strong stirring. The mixture is centrifuged for 45 min and the supernatant is eliminated. The desired 5'-OH-G^{IBu}-T-T-3'-*O*-Lev cyanoethyl phosphate triester trimer is obtained with a yield of 37% (calculated: 72 % per step) compared to the 5'-OH-T-T-3'-*O*-Lev. ^{31}P NMR (CD_3CN) d -1.59, -1.68, -1.71, -1.75. MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1149.60, m/z_{calc} = 1150.96. The spectrophotometric purity (87%) is determined by HPLC at 260 nm.

This low yield is due to the low solubility of the detritylated trimer in dichloromethane, acetonitrile.The trimer is soluble in methanol, and DMF.

Example 41

Synthesis of the dimer 5'-OH-T-A^{Bz}-3'-O-Lev cyanoethyl phosphate triester.

Coupling procedure of 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite with 5'-OH-A^{Bz}-3'-*O*-Lev using the PVP resin.

A solution of 5'-OH-A^{Bz}-3'-*O*-Lev (453 mg, 1 mmol) and 5'-*O*-DMTr-T-3'-cyanoethyl phosphoramidite (1.49 g, 2 mmol, 2 eq) in anhydrous acetonitrile (20 ml) is added to PVP resin (3 g, 10 mmol $pyrH^+$, 10 eq). The reaction is followed by reverse phase HPLC. After 5h the reaction is complete. The desired dimer 5'-*O*-DMTr-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphite triester is characterized

by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphite triester (d 140.48, 140.30) and of 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (d 8.76, 8.71).

Oxidation: The PVP resin is filtered off and the resulting solution is added to PS-$N(CH_3)_3^+$ IO_4^- (2 g, 5 mmol IO_4^-, 5 eq.). The reaction is followed by ^{31}P NMR and by reverse phase HPLC. The reaction is complete after 15 min. The desired dimer 5'-*O*-DMTr-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester is characterized by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-T-3'- cyanoethyl phosphate diester (^{31}P NMR (CD_3CN) d -3.01), 5'-*O*-DMTr-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester (d -1.58, -1.80), 5'-*O*-DMTr-T-3'-cyanoethyl hydrogenophosphonate (d 8.76, 8.71).

Detritylation: The PS-$N(CH_3)_3^+$ IO_4^- is filtered off and the solvent are evaporated. The crude is dissolved in 16 ml of CH_2Cl_2/CH_3OH (7/3) and cooled in an ice bath. To this solution is added 4 ml of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 45 min at 0°C. The reaction is stopped with 30 ml of a saturated solution of $NaHCO_3$. The aqueous phase is extracted three times with dichloromethane. The organic layer is separated, dried (Na_2SO_4) and evaporated under reduce pressure. The residue is dissolved in 10 ml of CH_2Cl_2/MeOH (4/1) and added to 100 ml of cooled diethyl ether drop by drop with a strong stirring. The mixture is centrifuged for 45 min and the supernatant is eliminated. The desired 5'-OH-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester dimer is obtained with a yield of 73 % (calculated: 90% per step) compared to the 5'-OH-T-3'-*O*-Lev. ^{31}P NMR (CD_3CN) d -1.77. MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 811.72, m/z_{calc} = 810.45. The spectrophotometric purity (96%) is determined by HPLC at 260 nm.

Example 42

Synthesis of the trimer 5'-OH-C^{Bz}-T-A^{Bz}-3'-O-Lev cyanoethyl phosphate triester.

Coupling procedure of 5'-*O*-DMTr-C^{Bz}-3'-cyanoethyl phosphoramidite with 5'-OH-T-A^{Bz}-3'-*O*-Lev using the PVP resin.

A solution of 5'-OH-T-A^{Bz}-3'-*O*-Lev (618 mg, 0.76 mmol) and 5'-*O*-DMTr-C^{Bz}-3'-cyanoethyl phosphoramidite (1.27 g, 1.52 mmol, 2 eq) in anhydrous acetonitrile (25 ml) and anhhydrous DMF (2.5 ml) is added to PVP resin (2.3 g, 7.6 mmol $pyrH^+$, 10 eq). The reaction is followed by reverse phase HPLC. After 2h30 the reaction is complete. The desired trimer 5'-*O*-DMTr-C^{Bz}-A^{Bz}-T-3'-*O*-

Lev cyanoethyl phosphite triester is characterized by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-C^{Bz}-A^{Bz}-T-3'-*O*-Lev trimer (phosphite triester linkage d 140.76, 140.65, 140.09, 140.03 ; phosphate triester linkage d -1.53, -1.57) and of 5'-*O*-DMTr-C^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.73).

Oxidation: The PVP resin is filtered off and the resulting solution is added to PS-$N(CH_3)_3^+$ IO_4^- (1.52 g, 3.8 mmol IO_4^-, 5 eq.). The reaction is followed by ^{31}P NMR and by reverse phase HPLC. The reaction is complete after 15 min. The desired trimer 5'-*O*-DMTr-C^{Bz}-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester is characterized by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-C^{Bz}-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester (d -1.54, -1.59, -162, -1.72), 5'-*O*-DMTr-C^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.74).

Detritylation: The PS-$N(CH_3)_3^+$ IO_4^- is filtered off and the solvent are evaporated. The crude is dissolved in 32 ml of CH_2Cl_2/CH_3OH (7/3) and cooled in an ice bath. To this solution is added 8 ml of a solution of benzene sulfonic acid 10% in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 1 h at 0°C. The reaction is stopped with 40 ml of a saturated solution of $NaHCO_3$. The aqueous phase is extracted three times with dichloromethane. The organic layer is washed with an aqueous solution of $Na_2S_2O_3$ 0.2 M. The organic layer is separated, dried (Na_2SO_4) and evaporated under reduce pressure. The residue is dissolved in 10 ml of CH_2Cl_2 and added to 100 ml of cooled diethyl ether drop by drop with a strong stirring. The mixture is centrifuged for 45 min and the supernatant is eliminated. The desired 5'-OH-C^{Bz}-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester trimer is obtained with a yield of 90% (calculated: 96% per step) compared to the dimer 5'-OH-T-A^{Bz}-3'-*O*-Lev. ^{31}P NMR (CD_3CN) d -1.75, -1.79, -1.87, -1.91. MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1257.53, m/z_{calc} = 1258.08. The spectrophotometric purity (89%) is determined by HPLC at 260 nm.

Example 43

Synthesis of the tetramer 5'-OH-G^{IBu}-C^{Bz}-T-A^{Bz}-3'-O-Lev cyanoethyl phosphate triester.

Coupling procedure of 5'-*O*-DMTr-G^{IBu}-3'-cyanoethyl phosphoramidite with 5'-OH-C^{Bz}-T-A^{Bz}-3'-*O*-Lev using the PVP resin.

A solution of 5'-OH-C^{Bz}-T-A^{Bz}-3'-*O*-Lev (866 mg, 0.69 mmol) and 5'-*O*-DMTr-G^{IBu}-3'-cyanoethyl phosphoramidite (1.14 g, 1.38 mmol, 2 eq) in anhydrous acetonitrile (25 ml) and anhhydrous DMF (2.5 ml) is added to PVP resin (2.1 g, 6.9 mmol $pyrH^+$, 10 eq). The reaction is followed by reverse phase HPLC. After

3h the reaction is complete. The desired tetramer 5'-*O*-DMTr-G^{IBu}-C^{Bz}-A^{Bz}-T-3'-*O*-Lev is characterized by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-G^{IBu}-C^{Bz}-A^{Bz}-T-3'-*O*-Lev tetramer (phosphite triester linkage d 141.86, 141.82, 141.76, 141.61, 140.69, 140.66, 140.61 ; phosphate triester linkage d -1.53, -1.58, -1.61, -1.64, -1.71, -1.82) and of 5'-*O*-DMTr-G^{IBu}-3'-cyanoethyl hydrogenophosphonate (d 9.01, 8.84).

Oxidation: The PVP resin is filtered off and the resulting solution is added to PS-$N(CH_3)_3^+$ IO_4^- (1.38 g, 3.45 mmol IO_4^-, 5 eq.). The reaction is followed by ^{31}P NMR and by reverse phase HPLC. The reaction is complete after 45 min. The desired tetramer 5'-*O*-DMTr-G^{IBu}-C^{Bz}-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester is characterized by ^{31}P NMR. The crude is a mixture of5'-*O*-DMTr-G^{IBu}-3'- cyanoethyl phosphate diester (^{31}P NMR (CD_3CN) d -2.69), 5'-*O*-DMTr-G^{IBu}-C^{Bz}-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester (d -1.38, -1.43, -1.53, -1.60, -1.64), 5'-*O*-DMTr-C^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 9.02, 8.38).

Detritylation: The PS-$N(CH_3)_3^+$ IO_4^- is filtered off and the solvent are evaporated. The crude is dissolved in 32 ml of CH_2Cl_2/CH_3OH (7/3) and cooled in an ice bath. To this solution is added 8 ml of a solution of benzene sulfonic acid 10 % in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 1 h 15 at 0°C. The reaction is stopped with 40 ml of a saturated solution of $NaHCO_3$. The aqueous phase is extracted three times with dichloromethane. The organic layer is washed with an aqueous solution of $Na_2S_2O_3$ 0.2 M. The organic layer is separated, dried (Na_2SO_4) and evaporated under reduce pressure. The residue is dissolved in 4 ml of CH_2Cl_2 and added to 100 ml of cooled diethyl ether drop by drop with a strong stirring. The mixture is centrifuged for 45 min and the supernatant is eliminated. The desired 5'-OH-G^{IBu}-C^{Bz}-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester tetramer is obtained with a yield of 83% (calculated: 94% per step) compared to the trimer 5'-OH-C^{Bz}-T-A^{Bz}-3'-*O*-Lev. ^{31}P NMR (CD_3CN) d -1.59, -1.69, -1.81. MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1708.44, m/z_{calc} = 1710.45. The spectrophotometric purity (80%) is determined by HPLC at 260 nm.

Example 44

Synthesis of the pentamer 5'-OH-A^{Bz}-G^{IBu}-C^{Bz}-T-A^{Bz}-3'-O-Lev cyanoethyl phosphate triester.

Coupling procedure of 5'-*O*-DMTr-A^{Bz}-3'-cyanoethyl phosphoramidite with 5'-OH-G^{IBu}-C^{Bz}-T-A^{Bz}-3'-*O*-Lev using the PVP resin.

A solution of 5'-OH-G^{IBu}-C^{Bz}-T-A^{Bz}-3'-*O*-Lev (982 mg, 0.57 mmol) and 5'-*O*-

DMTr-A^{Bz}-3'-cyanoethyl phosphoramidite (1.15 g, 1.15 mmol, 2 eq) in anhydrous acetonitrile (20 ml) and anhhydrous DMF (2.5 ml) is added to PVP resin (1.7 g, 5.7 mmol $pyrH^+$, 10 eq). The reaction is followed by reverse phase HPLC. After 3h the reaction is complete. The desired pentamer 5'-*O*-DMTr-A^{Bz}-G^{IBu}-C^{Bz}-A^{Bz}-T-3'-*O*-Lev is characterized by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-A^{Bz}-G^{IBu}-C^{Bz}-A^{Bz}-T-3'-*O*-Lev pentamer (phosphite triester linkage d 141.00, 140.78, 140.68, 140.16, 139.94 ; phosphate triester linkage d -1.36, -1.42, -1.46, -1.52, -1.57, -1.67, -1.70, -1.82) and of 5'-*O*-DMTr-A^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.76, 8.71).

Oxidation: The PVP resin is filtered off and the resulting solution is added to PS-$N(CH_3)_3^+$ IO_4^- (1.2 g, 2.87 mmol IO_4^-, 5 eq.). The reaction is followed by ^{31}P NMR and by reverse phase HPLC. The reaction is complete after 45 min. The desired pentamer 5'-*O*-DMTr-A^{Bz}-G^{IBu}-C^{Bz}-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester is characterized by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-A^{Bz}-3'- cyanoethyl phosphate diester (^{31}P NMR (CD_3CN) d -2.67), 5'-*O*-DMTr-A^{Bz}-G^{IBu}-C^{Bz}-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester (d -1.24, -1.39, -1.54, -1.66, -1.71), 5'-*O*-DMTr-A^{Bz}-3'-cyanoethyl hydrogenophosphonate (d 8.82).

Detritylation: The PS-$N(CH_3)_3^+$ IO_4^- is filtered off and the solvent are evaporated. The crude is dissolved in 50 ml of CH_2Cl_2, the solution is washed with 50 ml of an aqueous solution of $Na_2S_2O_3$ 0.2 M. The organic layer is separated, dried (Na_2SO_4) and evaporated under reduce pressure. The crude is dissolved in 16 ml of CH_2Cl_2/CH_3OH (7/3) and cooled in an ice bath. To this solution is added 4 ml of a solution of benzene sulfonic acid 10 % in CH_2Cl_2/CH_3OH (7/3). The solution is stirred 45 min at 0°C. 70 ml of CH_2Cl_2 and 10 ml of pyridine are added to the solution. The reaction is stopped with 70 ml of a saturated solution of $NaHCO_3$. The aqueous phase is extracted three times with 30 ml of CH_2Cl_2 and 5 ml of pyridine. The organic layer is separated, dried (Na_2SO_4) and evaporated under reduce pressure. The residue is dissolved in 5 ml of CH_2Cl_2/MeOH (4/1) and added to 100 ml of cooled diethyl ether drop by drop with a strong stirring. The mixture is centrifuged for 45 min and the supernatant is eliminated. The desired 5'-OH-A^{Bz}-G^{IBu}-C^{Bz}-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester pentamer is obtained with a yield of 85% (calculated: 95 % per step) compared to the tetramer 5'-OH-G^{IBu}-C^{Bz}-T-A^{Bz}-3'-*O*-Lev. ^{31}P NMR (CD_3CN) d -1.35, -1.42, -1.55, -1.63. MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 2179.40, m/z_{calc} = 2180.83. The spectrophotometric purity (84 %) is determined by HPLC at 260 nm.

Final deprotection of the pentamere :

20 mg (9 mmol) of the 5'-OH-A^{Bz}-G^{iBu}-C^{Bz}-T-A^{Bz}-3'-*O*-Lev cyanoethyl phosphate triester pentamer is dissolved in 5 ml of aqueous ammonia solution (30%). After 16 h at 55°C, the ammonia is evaporated. ^{31}P NMR (D_2O) d 0.26, 0.04, -0.03, -0.09. MALDI-TOF MS (negative mode, trihydroxyacetophenone as matrix) $[M+H]^-$ m/z_{exp} = 1486.29, m/z_{calc} = 1486.03. The spectrophotometric purity (74 %) is determined by HPLC at 260 nm.

H-PHOSPHONATE SYNTHESIS

Example 45

Synthesis of the dimer 5'-O-DMTr-dG^{iBu}-T-3'-O-Lev H-phosphonate

A solution of 5'-OH-T-3'-*O*-Lev (42.5 mg, 0.125 mmol) and of 5'-*O*-DMTr-dG^{iBu}-*H*-phosphonate TEA salt (120.7 mg, 0.150 mmol, 1.2 eq) in 2.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (430.0 mg, 2.1 mmol/g, 6.6 eq) that is suspended in 3.0 ml of the same solvent. The mixture is shaken for 1h 30 min at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 70%.
^{31}P NMR (CD_2Cl_2) ä 10.20, 8.99 ppm.
MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1025.72, m/z_{calc} = 1027.02.

The spectrophotometrical purity determined by HPLC is 98%.

Example 46

Synthesis of the dimer 5'-O-DMTr-dG^{iBu}-dC^{Bz}-3'-O-Lev H-phosphonate

A solution of 5'-OH-dC^{Bz}-3'-*O*-Lev (53.7 mg, 0.125 mmol) and of 5'-*O*-DMTr-dG^{iBu}-*H*-phosphonate TEA salt (120.7 mg, 0.150 mmol, 1.2 eq) in 2.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (430.0 mg, 2.1 mmol/g, 6.6 eq) that is suspended in 3.0 ml of the same solvent. The mixture is shaken for 1h 30 min at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed

by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 72%.

^{31}P NMR (CD_2Cl_2) ä 9.78, 9.16 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1113.16, m/z_{calc} = 1116.11.

The spectrophotometrical purity determined by HPLC is 99%.

Example 47

Synthesis of the dimer 5'-O-DMTr-dC^{Bz}-T-3'-O-Lev H-phosphonate

A solution of 5'-OH-T-3'-*O*-Lev (142.0 mg, 0.417 mmol) and of 5'-*O*-DMTr-dC^{Bz}-*H*-phosphonate TEA salt (120.0 mg, 0.500 mmol, 1.2 eq) in 4.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (830.0 mg, 2.1 mmol/g, 4.2 eq) that is suspended in 4.0 ml of the same solvent. The mixture is shaken for 1h at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 100%.

^{31}P NMR (CD_2Cl_2) ä 10.16, 8.72 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1020.21, m/z_{calc} = 1021.01.

The spectrophotometrical purity determined by HPLC is 99%.

Example 48

Synthesis of the dimer 5'-O-DMTr- dG^{iBu}-dA^{Bz} -3'-O-Lev H-phosphonate

A solution of 5'-OH-dA^{Bz}-3'-*O*-Lev (189.0 mg, 0.417 mmol) and of 5'-*O*-DMTr-dG^{iBu}-*H*-phosphonate TEA salt (402.0 mg, 0.500 mmol, 1.2 eq) in 4.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (830.0 mg, 2.1 mmol/g, 4.2 eq) that is suspended in 4.0 ml of the same solvent. The mixture is shaken for 1h at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The

organic fractions are collected, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 83%.

^{31}P NMR (CD_2Cl_2) ä 6.80, 6.70 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1140.30, m/z_{calc} = 1140.14.

The spectrophotometrical purity determined by HPLC is 85%.

Example 49

Synthesis of the dimer 5'-O-DMTr-T-T-3'-O-Lev H-phosphonate

A solution of 5'-OH-T-3'-*O*-Lev (142.0 mg, 0.417 mmol) and of 5'-*O*-DMTr-T-*H*-phosphonate TEA salt (354.8 mg, 0.500 mmol, 1.2 eq) in 4.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (830.0 mg, 2.1 mmol/g, 4.2 eq) that is suspended in 4.0 ml of the same solvent. The mixture is shaken for 1h at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 98%.

^{31}P NMR (CD_2Cl_2) ä 9.99, 8.55 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) no result

The spectrophotometrical purity determined by HPLC is 99%.

Example 50

Synthesis of the trimer 5'-O-DMTr-dG^{iBu}-T-T-3'-O-Lev H-phosphonate

Detritylation of 5'-*O*-DMTr-T-T-3'-*O*-Lev *H*-phosphonate

The *H*-phosphonate dimer 5'-*O*-DMTr-T-T-3'-*O*-Lev (115 mg, 0,123 mmol) is dissolved in 4.0 ml of CH_2Cl_2/MeOH (7:3) and cooled in an ice bath. To this solution 1.0 ml of a solution of 10% BSA (benzene sulfonic acid) in CH_2Cl_2/MeOH (7:3) is added drop wise under stirring and the progress of the reaction is monitored by TLC. After 15 min the solution is diluted with 20 ml of CH_2Cl_2 and then 0.4 g of poly(4-vinyl-pyridine) are added. The mixture is shaken 5 minutes and the resin is filtered off and washed with CH_2Cl_2. The product is purified by precipitation from CH_2Cl_2 with ether and dried under

vacuum. Yield 94%.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) no result

The spectrophotometrical purity determined by HPLC is 99%.

Coupling

A solution of 5'-OH-T-T-3'-*O*-Lev (73.2 mg, 0.116 mmol) and of 5'-*O*-DMTr-dG^{iBu}-*H*-phosphonate TEA salt (140.5 mg, 0.174 mmol, 1.5 eq) in 2.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (660.0 mg, 2.1 mmol/g, 12 eq) that is suspended in 4.0 ml of the same solvent. The mixture is shaken for 4h at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, dried over Na_2SO_4, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 55%.

^{31}P NMR (CD_2Cl_2) ä 10.22, 10.07, 9.73, 9.00, 8.84, 8.75, 8.59 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1312.50, m/z_{calc} = 1315.21.

The spectrophotometrical purity determined by HPLC is 93%.

Example 51

Synthesis of the tetramer 5'-O-DMTr-dC^{Bz}-dG^{iBu}-T-T-3'-O-Lev H-phosphonate

Detritylation of 5'-*O*-DMTr-dG^{iBu}-T-T-3'-*O*-Lev *H*-phosphonate

The *H*-phosphonate trimer 5'-*O*-DMTr-dG^{iBu}-T-T-3'-*O*-Lev (84 mg, 0,064 mmol) is dissolved in 4.0 ml of CH_2Cl_2/MeOH (7:3) and cooled in an ice bath. To this solution 1.0 ml of a solution of 10% BSA (benzene sulfonic acid) in CH_2Cl_2/MeOH (7:3) is added drop wise under stirring and the progress of the reaction is monitored by TLC. After 15 min the solution is diluted with 20 ml of CH_2Cl_2 and then 0.4 g of poly(4-vinyl-pyridine) are added. The mixture is shaken 5 minutes and the resin is filtered off and washed with CH_2Cl_2. The product is purified by precipitation from CH_2Cl_2 with ether and dried under vacuum. Yield 71%.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1012.07, m/z_{calc} = 1012.84.

The spectrophotometrical purity determined by HPLC is 83%.

Coupling

A solution of 5'-OH-dG^{iBu}-T-T-3'-*O*-Lev *H*-phosphonate (45.9 mg, 0.045 mmol) and of 5'-*O*-DMTr-dC^{Bz}-*H*-phosphonate TEA salt (54.3 mg, 0.068 mmol, 1.5 eq) in 1.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (260.0 mg, 2.1 mmol/g, 12 eq) that is suspended in 1.5 ml of the same solvent. The mixture is shaken for 3h and 30 minutes at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, dried over Na_2SO_4, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 75%.
^{31}P NMR (CD_2Cl_2) ä 11.58, 11.11, 10.52, 10.32, 10.14, 9.66, 9.40, 9.14, 8.99, 8.35, 7.40, 7.17, 6.99 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1690.10, m/z_{calc} = 1692.51.

The spectrophotometrical purity determined by HPLC is 92%.

Example 52

Synthesis of the dimer 5'-O-DMTr-dC^{Bz}-dA^{Bz} -3'-O-Lev H-phosphonate

A solution of 5'-OH-dA^{Bz}-3'-*O*-Lev (197.3 mg, 0.435 mmol) and of 5'-*O*-DMTr-dC^{Bz}-*H*-phosphonate TEA salt (400.0 mg, 0.500 mmol) in 4.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (830.0 mg, 2.1 mmol/g, 4.2 eq) that is suspended in 4.0 ml of the same solvent. The mixture is shaken for 1h at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 95%.
^{31}P NMR (CD_2Cl_2) ä 9.94, 8.94 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1132.14, m/z_{calc} = 1134.13.

The spectrophotometrical purity determined by HPLC is 96%.

Example 53

Synthesis of the trimer 5'-O-DMTr-dG^{iBu}-dC^{Bz}-dA^{Bz}-3'-O-Lev H-phosphonate

Detritylation of 5'-*O*-DMTr-dC^{Bz}-dA^{Bz}-3'-*O*-Lev *H*-phosphonate

The *H*-phosphonate dimer 5'-*O*-DMTr-dC^{Bz}-dA^{Bz}-3'-*O*-Lev (180 mg, 0,158 mmol) is dissolved in 4.0 ml of CH_2Cl_2/MeOH (7:3) and cooled in an ice bath. To this solution 1.0 ml of a solution of 10% BSA (benzene sulfonic acid) in CH_2Cl_2/MeOH (7:3) is added drop wise under stirring and the progress of the reaction is monitored by TLC. After 15 min the solution is diluted with 20 ml of CH_2Cl_2 and then 0.4 g of poly(4-vinyl-pyridine) are added. The mixture is shaken 5 minutes and the resin is filtered off and washed with CH_2Cl_2. The product is purified by precipitation from CH_2Cl_2 with ether and dried under vacuum. Yield 83%.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 831.91, m/z_{calc} = 831.75.

The spectrophotometrical purity determined by HPLC is 74%.

Coupling

A solution of 5'-OH- dC^{Bz}-dA^{Bz} -3'-*O*-Lev (109 mg, 0.131 mmol) and of 5'-*O*-DMTr-dG^{iBu}-*H*-phosphonate TEA salt (126.5 mg, 0.157 mmol, 1.2 eq) in 2.0 ml of CH_2Cl_2/py (1:1) is added to polystyrene-bound acid chloride (660.0 mg, 2.1 mmol/g, 6 eq) that is suspended in 3.5 ml of the same solvent. The mixture is shaken for 4h at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, dried over Na_2SO_4, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. Yield 50%.

^{31}P NMR (CD_2Cl_2) ä 10.24, 10.06, 9.96, 9.88, 9.44, 9.38, 9.33, 9.28 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} = 1516.14, m/z_{calc} = 1517.43.

The spectrophotometrical purity determined by HPLC is 74%.

Example 54

Synthesis of dimer 5'-O-DMTr-T-dG^{ibu}-3'-O-lev H-phosphonate

A solution of 5'-OH-dG^{ibu}-3'-*O*-Lev (435 mg, 1.0 mmol) and of 5'-*O*-DMTr-dT-

H-phosphonate TEA salt (850 mg, 1.2 mmol) in 7.0 ml of CH_2Cl_2/py (1:1, v/v) is added to polystyrene-bound acid chloride (1.5g, 2.4 mmol/g, 3 eq) that is suspended in 7.0 ml of the same solvent. The mixture is shaken for 2h15 at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The organic fractions are collected, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The isolated product is dried under vacuum. 914 mg Yield 89%.

^{31}P NMR (CD_2Cl_2) ä 12.63, 8.92 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} =1026.6, m/z_{calc} = 1027.0. The spectrophotometrical purity determined by HPLC is 96%.

Example 55

Synthesis of the trimer 5'-O-DMTr- C^{bz}-T-dG^{ibu}-3'-O-lev H-phosphonate

Detritylation of 5'-O-DMTr-T-dG^{lbu}-3'-O-lev H-phosphonate

The *H*-phosphonate dimer 5'-*O*-DMTr- T-dG^{lbu} -3'-*O*-Lev (430 mg, 0.420 mmol) is dissolved in 8.0 ml of CH_2Cl_2/MeOH (7:3, v/v) and cooled in an ice bath. To this solution 2.0 ml of a solution of 10% BSA (benzene sulfonic acid) in CH_2Cl_2/MeOH (7:3, v/v) is added drop wise under stirring and the progress of the reaction is monitored by TLC. After 20 min the solution is diluted with 50 ml of CH_2Cl_2 and then 0.8 g of poly(4-vinyl-pyridine) are added. The mixture is shaken 5 minutes and the resin is filtered off and washed with CH_2Cl_2. The product is purified by precipitation from CH_2Cl_2 with ether and dried under vacuum. 320mg Yield 100%. The spectrophotometrical purity determined by HPLC is 98%.

Coupling

A solution of 5'-OH- T-dGibu -3'-*O*-Lev (320 mg, 0.43 mmol) and of 5'-*O*-DMTr-dG^{IBu}-*H*-phosphonate TEA salt (413 mg, 0.52 mmol, 1.2 eq) in 4.5 ml of CH_2Cl_2/py (1:1, v/v) is added to polystyrene-bound acid chloride (1050 mg, 2.4 mmol/g, 5 eq) that is suspended in 4.5 ml of the same solvent. The mixture is shaken for 4h at room temperature until the disappearance of the monomers. The reaction is monitored by reverse phase HPLC. The resin is filtered, washed with CH_2Cl_2. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2.

The organic fractions are collected, dried over Na_2SO_4, the solvent is evaporated and the pyridine is eliminated by coevaporation with toluene. The product is purified by precipitation from CH_2Cl_2 with ether and dried under vacuum. 538 mg Yield 89%.

^{31}P NMR (CD_2Cl_2) ä 12.68, 12.46, 10.27, 9.46, 9.40, 9.11, 8.96, 8.92. ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} =1404.0, m/z_{calc} = 1404.3. The spectrophotometrical purity determined by HPLC is 94%.

EXAMPLE 56

Synthesis of the trimer 5'-O-DMTr- C^{bz}-T-dG^{ibu}-3'-O-lev S-phenyl phosphotriester

To a solution of 5'-O-DMTr- C^{bz}-T-dG^{ibu}-3'-O-lev H-phosphonate (110 mg, 0.078 mmol) in 2 ml of CH_2Cl_2/py (1:1), N-(phenylsulfanyl)phthalimide (80mg, 0.314 mmol, 4 eq) and Triethylamine (200 mL, 10 eq) were added. The reaction is stirred for 3h at room temperature. The solvent is evaporated. The product is purified by precipitation from CH_2Cl_2 with ether and dried under vacuum. 117 mg Yield 92%

^{31}P NMR signals between 24.09 and 25.76 ppm

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$ m/z_{exp} =1621.8, m/z_{calc} = 1620.6. The spectrophotometrical purity determined by HPLC is 87%.

EXAMPLE 57

Synthesis of the trimer 5'-O-DMTr- C^{bz}-T-dG^{ibu}-3'-O-lev S-cyanoethyl phosphotriester

The trimer 5'-O-DMTr- C^{bz}-T-dG^{ibu}-3'-O-lev H-phosphonate (118 mg, 0.084 mmol) is coevaporated twice with dry pyridine (2x2ml) and dissolved in 2 ml of CH_2Cl_2/py (1:1). To this solution is added successively triethylamine (6 mL, 0.5eq), trimethylsilyl chloride (100 mL, 0.84 mmol, 10 eq) and N-[(2-cyanoethyl)thio]phthalimide (78 mg, 0.336 mmol, 10 eq).

After 1h30 the reaction is complete. The pyridinium salt present in solution is removed by aqueous extraction and the aqueous phase is washed twice with CH_2Cl_2. The solvent is evaporated. The product is purified by precipitation from CH_2Cl_2 with ether and dried under vacuum. 106 mg Yield 80%

^{31}P NMR signals between 26.86 and 28.75 ppm.

MALDI-TOF MS (positive mode, trihydroxyacetophenone as matrix) $[M+H]^+$

m/z_{exp} =1574.6, m/z_{calc} = 1574.6. The spectrophotometrical purity determined by HPLC is 84%.

Example 58

GENERAL PROCEDURE FOR THE SYNTHESIS OF 5'-OH-3'-O-Lev CYANOETHYL PHOSPHOROTHIONOTRIESTER BASE PROTECTED DIMERS USING THE PHOSPHORAMIDITE METHOD ON 10 MMOL SCALE

Coupling procedure of 5'-*O*-DMTr-3'-cyanoethyl-phosphoramidite base protected nucleotide with 5'-OH-3'-*O*-Lev base protected nucleoside using the poly(4-vinylpyridinum *p*-toluenesulfonate) (Aldrich):

5'-OH-3'-*O*-Lev base protected nucleoside (10 mmol) and 5'-*O*-DMTr-3'-cyanoethyl-phosphoramidite base protected nucleotide (15 mmol, 1.5 eq) are dissolved in anhydrous dichloromethane (100 ml). The solution is transferred under argon in a flask containing the poly(4-vinylpyridinum *p*-toluenesulfonate) (100 mmol $PyrH^+$, 10 eq) and shaken. The reaction is followed by reverse phase HPLC and is usually complete between 1h30 and 2h30. The desired 5'-*O*-DMTr-3'-*O*-Lev cyanoethyl phosphite triester base protected dimer is characterized by ^{31}P NMR. The crude is a mixture of 5'-*O*-DMTr-3'-*O*-Lev cyanoethyl phosphite triester base protected dimer and of 5'-*O*-DMTr-3'-cyanoethyl *H*-phosphonate base protected nucleotide. The poly(4-vinylpyridinum *p*-toluenesulfonate) is filtered off, washed 3 times with 50 ml of CH_2Cl_2 and the solution is concentrated to 100 ml.

Sulfurization: To the resulting solution is added AMBERLYST A26 tetrathionate form (50 mmol $S_4O_6^{2-}$, 5 eq.), and the reaction mixture is shaken. The reaction is followed by reverse phase HPLC and ^{31}P NMR and is usually complete between 1h30 and 2h30. The crude is a mixture of 5'-*O*-DMTr-3'-*O*-Lev cyanoethyl phosphorothionotriester base protected dimer and 5'-*O*-DMTr-3'-cyanoethyl diester *H*-phosphonate base protected nucleotide. The resin AMBERLYST A26 tetrathionate form is filtered off and washed 3 times with 50 ml of CH_2Cl_2.

dimers	phosphite triester		phosphorothionotriester	
5'-*O*-DMTr-Nu-Nu-3'-O-Lev	RT (min)	^{31}P NMR d	RT (min)	^{31}P NMR d
A^{Bz}-A^{Bz}	17.81	140.64; 140.46	18.23	68.30 ; 68.24
C^{Bz}-A^{Bz}	19.09	140.48; 140.39	19.35, 19.61*	68.31 ; 68.26
G^{iBu}-T	15.95; 16.14*	143.50; 141.71	16.73; 17.01*	68.65 ; 68.19
G^{iBu}-A^{Bz}	16.86	140.34; 140.02	17.30; 17.56*	67.57 ; 67.41

* mixture of Sp and Rp diastereoisomeres

Detritytlation: To the previous solution is added 100 ml of MeOH so that the CH_2Cl_2/MeOH ratio is about 7/3. The mixture is cooled at 0°C. To the resulting solution is added 90 ml (56 mmol, 5.6 eq.) of a solution of benzene sulfonic acid 10 % in CH_2Cl_2/CH_3OH (7/3). The solution is stirred at 0°C. The detritylation is monitored by TLC and reverse phase HPLC. The reaction time is between 30 min and 1h. When the reaction is complete, 100 ml of H_2O is added to the mixture, the solution is shaken for 10 min at 0°C. Then, the reaction is stopped by stirring at 0°C for 10 min with 100 ml of a saturated solution of $NaHCO_3$. The solution is diluted with 300 ml of CH_2Cl_2. The organic layer is then washed up to 4 times with H_2O / saturated solution of $NaHCO_3$ in water (1/1), dried with Na_2SO_4 (50g) and evaporated. The crude product is dissolved in 50 ml CH_2Cl_2 and added dropwise to 1 l of diethylether at 0°C to give a white precipitate of the pure desired 5'-OH-3'-*O*-lev cyanoethyl phosphorothionotriester base protected dimer. The solid is filtered off, washed with 500 ml diethylether and dried under vacuum. The dimer is characterized by ^{31}P NMR and by MALDI-TOF. The spectrophotometric purity is determined by reverse phase HPLC at 260 nm.

Dimers	**MW (g/mol)**	**^{31}P-NMR**	**RT (min)**	**Dimers performed following this procedure**	
5'-OH-Nu-Nu-3'-*O*-Lev				Yield	HPLC-Purity
A^{Bz}-A^{Bz}	939.91	68.11; 68.06*	10.85	91% (9.5 g)	91%
C^{Bz}-A^{Bz}	915.88	68.03; 67.80*	11.32; 11.46*	94% (8.7 g)	91%
G^{iBu}-T	808.77	68.48 ; 68.09*	9.72	91% (7.4 g)	91%
G^{iBu}-A^{Bz}	921.89	68.02 ; 67.80*	10.32 ; 10.68*	98% (10.3 g)	92%

*mixture of Sp and Rp diastereoisomeres

HPLC-Gradient [column: Macherey- Nagel Nucleosil 100-5 C18]:

0 to 5 min --> 10% of CH_3CN to 40% of CH_3CN (in TEAAc 50 mM)

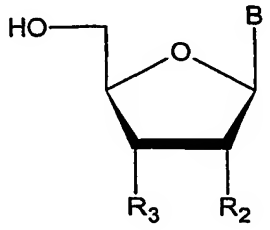
5 to 20 min --> 40% of CH_3CN to 80% of CH_3CN (in TEAAc 50 mM)

20 to 25 min --> 80% of CH_3CN to 100% of CH_3CN (in TEAAc 50 mM)

Claims

1. A method for preparing an oligonucleotide comprising the steps of

a) providing a 3'-protected compound having the formula:



wherein

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4'-O2'methylen linkage

R_3 is OR'_3, NHR''_3, $NR''_3R'''_3$, a 3'-protected nucleotide or a 3'-protected oligonucleotide,

R'_3 is a hydroxyl protecting group,

R''_3, R'''_3 are independently an amine protecting group,

b) reacting said compound with a nucleotide derivative having a 5'-proctection group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bond

c) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequence

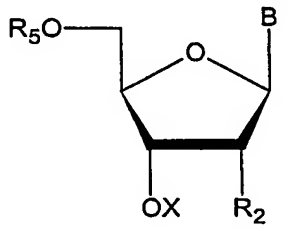
c1) capping preferably by reacting with a solid supported capping agent

c2) oxidizing preferably by reacting the oligonucleotide with a solid supported oxidizing reagent

d) removing the 5'-protection group.

2. The method of claim 1, wherein the step d) is effected by treatment with a solid supported agent or removing the 5'-protection group with a removal agent followed by addition of a solid supported scavenger or followed by extraction.

3. The method of claim 1 or 2, wherein the nucleotide derivative having a 5'-proctection group of step b) has the following formula:



wherein

X is a P(III)-function

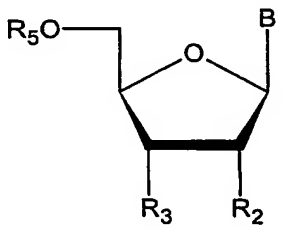
B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4'-O2'methylen linkage

R_5 is a hydroxyl protecting group, a 5'-protected nucleotide or a 5'-protected oligonucleotide.

4. A method for preparing an oligonucleotide comprising the steps of

a) providing a 5'-protected compound having the formula:



wherein

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4'-O2'methylen linkage

R_3 is OH, NH_2

R_5 is a hydroxyl protecting group, a 5'-protected nucleotide or a 5'-protected oligonucleotide

b) reacting said compound with a nucleotide derivative having a 3'-

proctection group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bond

c) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequence

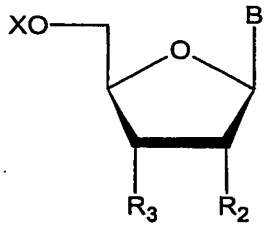
c1) capping, preferably by reacting with a solid supported capping agent

c2) oxidizing, preferably by reacting the oligonucleotide with a solid supported oxidizing reagent

d) removing the 3'-protection group.

5. The method of claim 4, wherein step d) is effected by treatment with a solid supported agent or removing the 3´-protection group with a removal agent followed by addition of a solid supported scaenger or followed by extraction.

6. The method of claim 4 or 5, wherein the nucleotide derivative having a 3'-proctection group has the following formula:



wherein

X is a P(III)-function

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4´-O2´methylen linkage

R_3 = OR'_3, NHR''_3, $NR''_3R'''_3$, a 3'-protected nucleotide or a 3'-protected oligonucleotide,

R'_3 is a hydroxyl protecting group,

R''_3, R'''_3 are independently an amine protecting group,

R'_3 is a hydroxyl protecting group, a 3'-protected nucleotide or a 3'-protected oligonucleotide

7. The method of any one of claims 1 to 5, comprising the further step of

e) repeating steps a) to d) at least once.

8. The method of any one of claims 1 to 6, wherein the nucleotide derivative of step b) is a phosphoramidite or a H-phosphonate.

9. The method of any one of steps 1 to 8, wherein the solid supported activator of step b) is selected from the group consisting of a solid support bearing a pyridinium salt, a cation exchange solid support with an optionally substituted pyridinium, a cation exchange solid support with an optionally substituted imidazolium salt, a solid support bearing an optionally substituted azole (imidazol, triazole, tetrazole), a salt of a weak base anion exchange resin with a strong acid, a weak cation exchange resin (carboxylic) in its protonated form, a solid support bearing an optionally substituted phenol, a solid support bearing a carboxylic acid chloride/bromide, a sulfonic acid chloride/bromide, a chloroformate, a bromoformate, a chlorosulfite, a bromosulfite, a phosphorochloridate, a phosphorbromidate and a solid support bound carbodiimide.

10. The method of any one of claims 1 to 9, wherein the solid supported oxidizing reagent is selected from the group consisting of solid supported periodates, permanganates, osmium tetroxides, dichromates, hydroperoxides, substituted alkylamine oxides, percarboxylic acid and persulfonic acid.

11. The method of any one of claims 1 to 10, wherein the oxidizing is a sulfurization.

12. The method of claim 11, wherein the solid supported oxidizing reagent is selected from the group consisting of a solid supported tetrathionate, a solid supported alkyl or aryl sulfonyl disulfide, a solid supported optionally substituted dibenzoyl tetrasulfide, a solid supported bis(akyloxythiocarbonyl)tetrasulfide, a solid supported optionally substituted phenylacetyl disulfide, a solid supported N-[(alkyl or aryl)sulfanyl] alkyl or aryl substituted succinimide and a solid supported (2-pyridinyldithio) alkyl or aryl.

13. The method of any one of claims 1 to 12, wherein the solid supported capping agent is a solid supported activated acid, preferably a carboxylic acid chloride, carboxylic acid bromide, azolide, substituted azolide, anhydride or chloroformate or phosphorochloridate, or a solid supported phosphoramidite, or a solid supported H-phosphonate monoester.

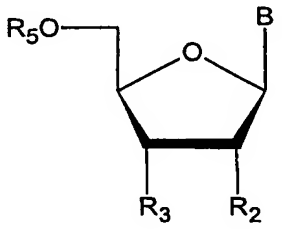
14. The method of any one of claims 1 to 13, wherein the 5′-protection is a dimethoxytrityl group (DMTr) or a monomethoxytrityl group (MMTr) and the solid supported agent of step d) is an cationic ion exchanger resin in the H^+ form or solid supported ceric ammonium nitrate.

15. The method of any one of claims 1 to 14, wherein the 3′-protection is a silyl group and the solid supported agent of step d) is an anionic ion exchanger resin in the F-form or the 3′-proctection is levulinic acid and the solid supported agent of step d) is a solid supported hydrazine or a solid supported hydrazinium.

16. Use of a solid supported sulfurization agent consisting of solid supported amine and a tetrathionate having the formula S_4O_6 or a cyanoethylthiosulfate ($NC\text{-}CH_2\text{-}CH_2\text{-}S\text{-}SO_3^-$) for sulfurization of oligonucleotides.

17. A method for preparing an oligonucleotide comprising the steps of

a) providing a compound having the formula:



wherein

B is a heterocyclic base

R_2 is H, a protected 2′-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4′-O2′methylen linkage

and

R_3 is OR'_3, NHR''_3, $NR''_3R'''_3$,

a protected nucleotide or a protected oligonucleotide and R_5 is a P(III) function

R'_3 is a hydroxyl protecting group,

R''_3, R'''_3 are independently an amine protecting group,

or

R_5 is a hydroxyl protecting group, a protected nucleotide or a protected oligonucleotide and R_3 is a P(III) function

b) reacting said compound with a nucleotide derivative having a 3' or 5'-free OH-group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bond

c) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequence

c1) capping by reacting with a solid supported capping agent

c2) oxidizing by reacting the oligonucleotide with a solid supported oxidizing reagent

d) removing the 3' or 5'-protection group.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/08447

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X Y	WO 99 52926 A (TRIAS JOAQUIM ;ROSENOW CARSTEN (US); VERSICOR INC (US); YOUNG DENN) 21 October 1999 (1999-10-21) the whole document, in particular: claims 31-40; page 5, lines 1-6; Figure 15	1,3,4, 6-9,14, 17 1-9,17
X Y	WO 01 51532 A (UNIV MICHIGAN) 19 July 2001 (2001-07-19) the whole document, in particular: claims 39-63; Figure 21	1,3,4, 6-9,14, 17 1-9,17

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance
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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 October 2003	03/11/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fitz, W

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/08447

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	WO 99 62922 A (MANOHARAN MUTHIAH ;RAVIKUMAR VASULINGA T (US); SANGHVI YOGESH (US)) 9 December 1999 (1999-12-09) the whole document, in particular: claims 10,11; pages 65-66, example 5	1-9,17
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PCT/EP 03/08447

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